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RETICULO-ENDOTHELIOSIS

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Reticulo-endotheliosis is a generalized hyperplasia of the cells of the reticulo-endothelial system. In surveying the literature on the subject one finds such a variety of opinions as to the etiology and nature of the condition and even as to the exact definition of the term that one must indeed be courageous who would take a strong position as to the significance of such a reaction. On the other hand, it is only by the presentation of cases studied as accurately as possible that a clear picture may finally emerge from the present confusion, and it is with this in mind that this report is offered.

REPORT OF A CASE

A 36 year old white housewife was admitted to the hospital June 20, 1935. Her chief complaint was weakness. In June 1934 she had chills. Her physician found albumin in the urine and placed her on a raw vegetable, low salt diet. Weakness was profound, and there was some edema of the ankles. After a month in bed the patient was up, but weakness persisted. After she had followed the diet for ten months, she was placed on a diet of milk. At this time, all the teeth were extracted. During the two months before admission to the hospital, epistaxis occurred three or four times each day. Dyspnea developed. Additional complaints during these months were of scotomas, occasional night sweats, palpitation, severe constipation, frequency of micturition (from five to seven times daily) and nocturia (from six to eight times). The menses ceased, but profuse leukorrhea was persistent.

The only previous illnesses were measles and mumps in childhood. The social and family histories were irrelevant.

Examination showed a well developed and well nourished woman with some evidence of loss in weight. There were pallor of the skin and mucous membranes with slight icterus, numerous retinal hemorrhages of various sizes and shapes, blurring of the margins of the optic disks, dilatation of the retinal veins, injection of the nasal mucous membranes and absence of all the teeth. There were scattered fine râles at the base of the right lung posteriorly. The heart was enlarged, the apex extending 115 mm. to the left of the midsternal line, and there were a localized systolic thrill and murmur at the apex. The systolic blood pressure was 134 mm. of mercury; the diastolic, 50. The liver extended to just below the level of the umbilicus and the spleen to 8 cm. below the costal margin. There was no

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enlargement of lymph nodes. The deep reflexes were hypo-active, and there were no abnormal reflexes.

The urine had a specific gravity of 1.011, an acid reaction, 0.3 per cent albumin and from 2 to 8 granular casts per ten high power fields. Three tests of the urine done during her residence in the hospital were positive for Bence-Jones protein. The twenty-four hour loss of albumin totaled 9 Gm. in one instance. The blood showed 25 per cent hemoglobin (Tallqvist), 950,000 erythrocytes and 4,300 leukocytes per cubic millimeter with 47 per cent neutrophils, 4 per cent metamyelocytes and 49 per cent lymphocytes of all types. The platelet count was 40,000. The fragility of the erythrocytes was normal. The icterus index was 8. The Wassermann reaction of the blood was negative. The blood sugar was 104 mg. per hundred cubic centimeters of blood; the nonprotein nitrogen, 25 mg. The total serum protein was 7.04 Gm. per hundred cubic centimeters of blood; the albumin, 2.95 Gm., and the globulin, 4.09 Gm. Two days later the albumin was 4.05 and the globulin 3.75 Gm. Gastric analysis showed a total acid of 70 degrees and a free acid of 40 degrees. Two blood cultures during her residence in the hospital were negative. A bedside roentgenogram showed cardiac enlargement and changes in both lung fields indicative of congestion.

On the day of admission the temperature was 101 F., and remittent fever was subsequently observed. After the first few days, the signs in the chest became more marked, the respirations more rapid, the pulse weaker, the abdomen distended. A diagnosis of bronchopneumonia appeared to be confirmed by a second roentgenogram. The usual therapeutic measures were enforced, and the patient's condition improved temporarily. Liver extract, derived from 100 Gm. of liver, was administered intramuscularly at three day intervals. The anemia and leukopenia persisted. A week after admission the blood showed 24 per cent hemoglobin (Haden-Hausser), 1,000,000 erythrocytes, and 6,225 leukocytes per cubic millimeter. The differential count gave percentages as follows: neutrophils, 56.6; basophils, 0.4; small lymphocytes, 27.4; young lymphocytes, 0.4; large lymphocytes, 4; large mononuclears, 2.8; neutrophilic myelocytes, 1.6; unclassified, 3; primitive cells, 1.4, and pathologic cells, 2. There was marked variation in the size and shape of the erythrocytes. The volume index was 1.04. Occasional normoblasts were seen. The reticulocytes numbered 7.1 per cent. The platelets were slightly reduced. In the majority of the neutrophils the nuclei had two, three or four lobes.

A sternal puncture with removal of marrow was done on the sixteenth hospital day, and examination of this showed percentages as follows: neutrophils, 67.5; eosinophils, 0.5; basophils, 0.5; small lymphocytes, 18; young lymphocytes, 3; large mononuclears, 2.5; primitive cells, 2.5; unclassified cells, probably monocytes, 2.5, and cells that appeared to be plasma cells, 1. The primitive cells were not perfect but were drawn out, and in some instances the cytoplasm was ruptured. The nuclei were rather reticular, with one or two nucleoli. The neutrophils were of all ages. There were many platelets in the smear.

It was thought that the patient had aleukemic leukemia, but this diagnosis was not established.

Despite symptomatic measures and the administration of large doses of iron and liver extract, the anemia persisted. After twenty-three days the patient was given a transfusion of 550 cc. of whole blood. Three days later a transfusion of 500 cc. was given. The hemoglobin rose to 55 per cent; the erythrocytes, to 3,050,000. Roentgenograms of the skull and several long bones at this time revealed nothing to suggest multiple myeloma. There was subjective and objective improvement for a few days, then decline with accumulation of fluid in the pleural and abdominal cavities. The temperature rose to 104 F. or higher each afternoon. The icterus index rose to 30. At this time the second blood culture was negative.

On the thirty-fourth day in the hospital the patient developed bilateral otitis media, and myringotomy was done. Suddenly, while being turned, the patient died on the thirty-fifth day in the hospital.

Autopsy.—The pertinent gross findings were as follows: Petechiae were seen over the right side of the thorax and on the anterior abdominal surface. Moderate jaundice was present over the entire body. A large amount of bile-stained fluid under pressure was present in the peritoneal cavity; the right pleural cavity contained 550 cc. of slightly bloody bile-stained fluid; the left cavity contained 150 cc.

Several small areas of subpleural hemorrhage were noted. There was bilateral localized atelectasis, roughly proportional on the two sides to the amounts of fluid present.

The spleen weighed 900 Gm., and localized areas of acute inflammatory exudate were present on the capsular surface. Cutting revealed a surface which was medium red, fairly firm and bulging. The malpighian corpuscles were of the usual appearance, and the connective tissue trabeculae were not increased.

The liver weighed 3,350 Gm. The capsular surface was not remarkable, but the cut surface was bulging, opaque and congested. Many opaque yellow areas about 1 mm. in diameter and slightly larger opaque gray areas could be seen.

The kidneys weighed 290 and 310 Gm., respectively. Their capsules stripped easily, leaving a surface for the most part smooth; several raised areas, however, some hemorrhagic and some gray and medullary, were seen. These measured 2.5 cm. or less in diameter. They extended into the substance of the kidneys along the cortical vascular markings.

The vertebral bone marrow was pale, but no other change was evident.

The pelvic organs were surrounded by an increased amount of connective tissue, in which there were many yellow opaque streaks.

Microscopic Observations.—Lymph Nodes: The normal architecture had largely disappeared and was replaced by a reticular network, on which lay cells of various types. These were for the most part larger than mature lymphocytes and gave the impression of being primitive cells of the lymphoid series. Scattered lymphocytes were seen. Giant cells were seen in close relation to the sinuses, some being in the lumen and others along the borders, particularly along the hilar aspect of the peripheral sinus (fig. 1 *A*). These giant cells contained one or more nuclei, some of which were round, while others were indented or lobulated in a bizarre manner, and many were pyknotic. The distribution of chromatin varied but was fairly even in most instances. Occasional metachromatic nucleoli were seen. The cytoplasm was azurophilic and vacuolated in some cells, but no granules were found. A stain for reticulum revealed that these giant cells were in most cases intimately related to the reticular network of the node.

Spleen: The malpighian corpuscles were considerably decreased in size, and there was early fibrosis at their periphery. In the pulp an increased number of reticulo-endothelial cells was present, and similar cells were seen also in the venous sinuses (fig. 1 *B*). It is impossible to say with certainty whether the lining endothelium of the sinuses participated in the hyperplasia, but the impression was gained that some of the large round cells in the lumens arose from the endothelial lining. For the most part, however, the endothelium was fairly flat. Distributed throughout the spleen but especially about the malpighian corpuscles were occasional giant cells, similar to those seen in the lymph nodes. The pulp and sinuses were congested with red cells. An occasional eosinophil was present in the pulp. The reticulum as revealed by staining with silver did not appear increased.

Liver (fig. 2*A*): The architecture was considerably distorted; the columns of liver cells were atrophic and in some areas altogether absent, leaving merely a

network of reticulum, in which were cells of various characters. The liver cells were chiefly swollen and granular, though some had undergone fatty change. In the sinusoids there were many nucleated cells of various types; some were round or oval, and some were elongated and similar to Kupffer cells. The majority of these cells were similar to the hyperplastic reticulo-endothelial cells seen in the spleen. Here and there, apparently springing from Kupffer cells, were giant cells similar to those found in the spleen. The reticulum seemed coarser than in a normal liver.

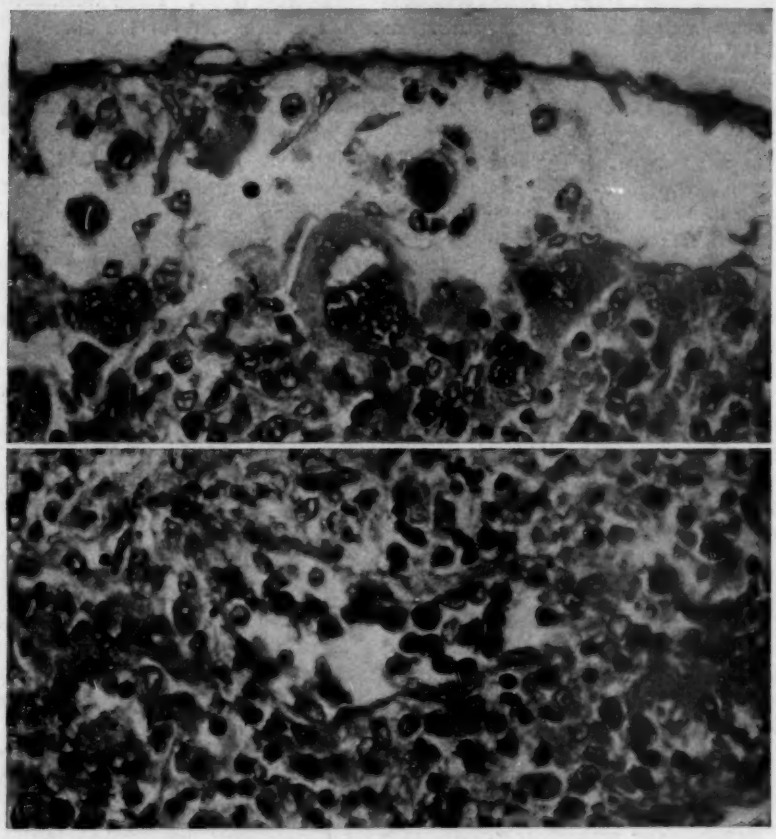

Fig. 1.—*A*, an area of a lymph node showing a peripheral sinus with giant cells scattered along the central border. Zeiss apochromatic 8 mm.; Homal I; × 500. *B*, an area of the spleen showing a venous sinus containing large nucleated cells. Some swelling of the lining endothelium is seen. Zeiss apochromatic 8 mm.; Homal I; × 500.

Bone Marrow: The normal architecture was lost, and the usual blood-forming centers were replaced by cells of various types (fig. 2*B*). These were distributed apparently in a rather haphazard manner. Large cells with scanty cytoplasm were seen, and these appeared to be primitive blood cells in the blast stage. Groups of nucleated red cells were present, and there were many plasma cells. The remaining

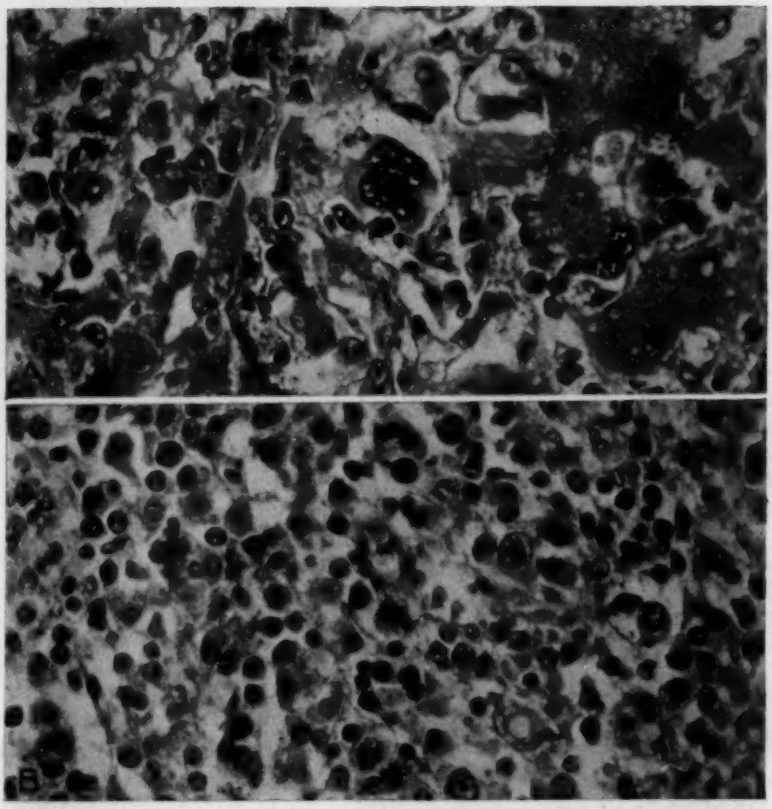

Fig. 2.—*A*, an area of the liver showing a giant cell. Note its position in a sinusoid and its processes resembling those of a Kupffer cell. Zeiss apochromatic 8 mm.; Homal I; × 500. *B*, bone marrow. Zeiss apochromatic 8 mm.; Homal I; × 500.

Fig. 3.—A clump of crystals in a phagocyte from the capsule of the adrenal gland. Zeiss apochromatic 4 mm.; Homal III; × 600.

type was a cell with fairly abundant agranular azurophilic cytoplasm and a round, oval or kidney-shaped nucleus. This resembled a myelocyte except that no granules were seen and the chromatin was distributed in definite clumps instead of in a network. Such cells could not be placed definitely in the hematopoietic series but were regarded as abnormal cells probably derived from that series.

Besides these cells there were also many clumps of crystals, situated within phagocytes (fig. 3). These crystals stained strongly with eosin, basic and acid fuchsin, and trinitrophenol (Van Gieson's stain). They were unstained by congo red and were not metachromatic with methyl violet. Acids and alkalis of moderate strength did not dissolve them, nor did the fat solvents. Considerable fibrosis was present.

Connective Tissue: In many parts of the body, but especially in the capsule of the adrenal gland, large phagocytes were found containing crystals similar to those just described. Giant cells similar to those found in the spleen, liver and lymph nodes were also found scattered throughout the connective tissue of the body.

Summary of Pathologic Findings.—The gross findings were: enlarged liver; greatly enlarged spleen (no enlargement of lymph nodes); pale vertebral bone marrow; ascites and hydrothorax; jaundice; chronic catarrhal gastritis.

The microscopic observations were: reticulo-endothelial hyperplasia involving the liver (Kupffer cells), spleen, lymph nodes, bone marrow and the reticulo-endothelial cells of the connective tissue, with the formation of bizarre giant cells; crystals of unknown composition in phagocytes in widely distributed regions.

COMMENT

Several striking features are immediately evident in this case. In the first place the reaction was sharply limited to the reticulo-endothelial system. Other elements, so far as we could ascertain, were affected only indirectly as they were crowded by this reticulo-endothelial hyperplasia. Two points differentiate this case from those of reticulo-endothelial hyperplasia occurring in response to a recognized infectious or toxic agent, namely (1) the absence of a known cause and (2) the presence of atypical giant cells. These features, then, put the condition in the class of idiopathic reticulo-endotheliosis, and our thought in regard to this will be expressed later.

One group of clinical signs is especially worthy of note. On admission there were multiple retinal hemorrhages, the urine contained 0.3 per cent albumin, and the albumin-globulin ratio in the blood was reversed. This syndrome led us to suspect a rather severe renal lesion. Such a lesion was not found; the kidneys were the site only of rather severe degenerative lesions, which could easily have been the result of the severe anemia combined with the toxicity of the terminal infection. The question of multiple myeloma was raised because of the presence of Bence-Jones protein in the urine but no evidence for this lesion was determined. The marrow, however, was hyperactive, and many abnormal cells were found in it. It is conceivable that such a lesion might produce a change in the metabolism similar to that found in multiple myeloma, and therefore give rise to this urinary phenomenon.

Another point of interest lies in the reaction of the lymph nodes. Because the clinical evidence pointed toward a disease of leukemic type, enlarged nodes were particularly sought at the autopsy. Nevertheless none could be found, and a few lymph nodes of normal appearance were removed and preserved with the aorta. In spite of this absence of gross abnormality a striking microscopic change was present, consisting of the replacement of normal lymphoid tissue by abnormal cells.

The crystals described in many of the phagocytes are extremely puzzling. Maximow [1] showed similar crystals in an illustration, but we can find no reference to them in the text of his monograph. The legend of the illustration refers to them as "eosinophile crystalline inclusions." One can only wonder whether the presence of Bence-Jones protein in the urine might have been related to the presence of these crystals.

The question as to the type of disease represented here is naturally paramount. The case seemed clinically to be one of aleukemic leukemia of the myelogenous type (aleukemic myelosis), but this diagnosis could not be confirmed.

Consideration of the pathologic picture brings up the discussion of reticulo-endotheliosis. A review of the rather confused literature is not necessary here, as this has been given recently by Foot and Olcott.[2] A few observations, however, should be made.

In the first place, the general tendency seems to be to regard reticulo-endotheliosis as a disease comparable to myelosis and lymphadenosis. The latter are well defined and divided from hyperplasias arising merely from increased functional demand. On the other hand, it is obvious from an examination of the literature that no such division is possible at present in the case of hyperplasia of the reticulo-endothelial system, and hence this should be regarded at present as a symptomatic phenomenon rather than as a pathologic entity.

Many diseases cause localized reticulo-endothelial hyperplasia; in fact, almost any chronic infection may be accompanied by such a reaction. Some diseases, such as tuberculosis or brucella infection (Sprunt and McBryde [3]), may give rise to more or less generalized stimulation of the reticulo-endothelial system.

A second type is generally known as an error in metabolism—namely, the lipoid disorders, such as Niemann-Pick's disease and Hand-Schüller-Christian's xanthomatosis. The condition in question is not considered as having much in common with such entities. The name "nonlipoid

1. Maximow, A. A.: Bindegewebe und blutbildende Gewebe, in Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 2, pt. 1, p. 262.

2. Foot, N. C., and Olcott, C. T.: Am. J. Path. **10**:81, 1934.

3. Sprunt, D. H., and McBryde, A.: Arch. Path. **21**:217, 1936.

histiocytosis" as applied by Foot and Olcott to generalized reticulo-endothelial hyperplasia reflects a possible relationship between the lipoid disorders and the type of disorder under discussion.

The third type is related to the leukemias. Changes similar to those occurring in the present case are present in cases of so-called monocytic leukemia (better known as stem cell leukemia since the abnormal cells in the blood are not typical mature monocytes). When definite changes are present in the blood picture the diagnosis is fairly clear. When, on the other hand, there are no distinctive changes in the blood picture, it is a problem to determine into what group of lesions the condition should fall. In analyzing the present case, the following points bring us to the conclusion that this is an aleukemic reticulo-endotheliosis.

First, no chronic infection could be ascertained. Bronchopneumonia developed during the patient's stay in the hospital, but acute bronchopneumonia is hardly to be regarded as the cause of such a reaction. Otitis media also developed, but this was apparently only the day before death and could not have caused such profound changes. If the cause was infection, this surely was extremely atypical and obscure.

Second, the disturbance was so sharply limited to the reticulo-endothelial system as to resemble a system disease. The bone marrow was the site of especially severe involvement—a fact pointing toward a lesion of leukemic character.

Third, the giant cells in the liver were found in the sinusoids, in close association with the reticular framework of the liver. Thus the evidence points definitely to the conclusion that these giant cells arose from the Kupffer cells in situ—a striking instance of the selective action of a pathogenic agent on a specific type of cell.

Giffin and Watkins [4] reported two cases clinically similar to the present one. In both patients weakness, anemia and splenomegaly were prominent. A diagnosis of splenic anemia was made in each case, but splenectomy resulted in no improvement. In these cases the spleen was the only tissue available for study; one patient was living at the time of their publication, and the second had died, but no permission for autopsy was granted. The microscopic examination of the spleen revealed a great overgrowth of reticulo-endothelial cells, and some evidence pointing to the escape of these cells into the blood was presented. On reviewing the blood smears after examination of the spleens of their patients, they were able to find a few "reticulo-endothelial monocytes" and reticulo-endothelial cells which had been regarded as normal monocytes on routine examination. They therefore called the disease "subleukemic reticulo-endotheliosis" and believed it to be closely related to monocytic or stem cell leukemia.

4. Giffin, H. Z., and Watkins, C. H.: Am. J. M. Sc. **188**:761, 1934.

Last, the presence of numerous atypical giant cells points not to any ordinary infectious cause but toward an agent which interferes with the proper growth and division of reticulo-endothelial cells. The presence of these giant cells naturally suggests Hodgkin's disease; this, however, is easily excluded by the absence of the other changes characteristic of this disease.

The logical classification of the reticulo-endothelial hyperplasias must await the adequate description of a larger number of cases and further contributions to the knowledge of the obscure types.

SUMMARY AND CONCLUSIONS

Reticulo-endothelial hyperplasias may be divided into three types: (*a*) infectious, including tuberculosis, brucella infections and many others; (*b*) metabolic, including Gaucher's splenomegaly, Hand-Schüller-Christian's xanthomatosis and Niemann-Pick's disease, and (*c*) leukemic and aleukemic types, evidently related to so-called monocytic or stem cell leukemia. The case presented is apparently one of aleukemic reticulo-endotheliosis.

STUDIES OF SERUM ELECTROLYTES

XI. BLOOD CHANGES FOLLOWING DEATH FROM CONTACT WITH ALTERNATING CURRENT OF HIGH INTENSITY

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The literature concerning electrical injuries and the effect of electrical currents on the various organs of the body has been reviewed by Jaffé,[1] Langworthy,[2] Langworthy and Kouwenhoven [3] and others. The experimental studies have dealt principally with the mechanisms of death and the effect of the current on the central nervous system. Although these studies indicate that death from electricity is due to paralysis of the heart or of the respiratory center or both, the ultimate cause of death is still in dispute. Postmortem examinations have not been very revealing since no characteristic visceral lesions have been found. With the exception of frequent reports of the release of hemoglobin into the body fluids as evidenced by hemoglobinuria and staining of the serum, relatively few observations of the blood have been made following contact of the body with an electrical current.

Kawamura [4] subjected guinea-pigs to direct and alternating currents and made observations on the blood for changes in the coagulation time. From his studies he concluded that alternating currents decreased the coagulation time and that direct currents increased it. Langworthy and Kouwenhoven [3] studied the changes in the morphology of the blood cells in rats following electrical shock. They observed no constant abnormality in the erythrocytes but obtained leukocytosis with an increase in the percentage of polymorphonuclear cells within two and one-half hours after the injury. Nasset, Bishop and Warren [5] applied a high frequency current (diathermy) to anesthetized dogs and

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1. Jaffé, R. H.: Arch. Path. **5**:837, 1928.
2. Langworthy, O. R.: J. Exper. Med. **51**:943, 1930.
3. Langworthy, O. R., and Kouwenhoven, W. B.: Am. J. Hyg. **16**:625, 1932.
4. Kawamura, J.: Ztschr. f. d. ges. exper. Med. **12**:168, 1921.
5. Nasset, E. S.; Bishop, F. W., and Warren, S. L.: Am. J. Physiol. **96**:439, 1931.

observed that when the temperature of the body reached 41 to 42 C. (105.8 to 107.6 F) the concentrations of sugar and carbon dioxide in the blood were diminished, the concentration of nonprotein nitrogen was increased and that of chloride remained essentially unchanged.

Despite the fact that blood is one of the best conductors in the body, practically no chemical studies of the blood have been made following contact of any person with a high intensity current. Therefore, the opportunity to obtain blood before and after the electrocution of condemned criminals seemed to us to justify such an investigation.

METHODS

Before the electrocution of three persons blood was removed from a cubital vein of each and defibrinated under oil. A portion of this blood was centrifugated, and the serum was removed under oil. Blood was obtained from two of the subjects (T and M) from thirty to forty-five minutes before death and from the other (W) about four hours before death. Within five minutes after the current had been turned off blood was collected in a similar manner from the right side of the heart. The time required for the complete separation of the fibrin in each sample was approximately ten minutes.

A 60 cycle alternating current of 2,000 volts and from 12 to 14 amperes was applied to each person for a period of three minutes.

The details of the methods used in the various analyses have been recorded in previous papers in this series.[6] Analyses for sugar were begun immediately after the blood was collected in order to prevent glycolysis.

RESULTS

The results of the analyses made on the whole blood and serum are given in tables 1 and 2.

The serums obtained after electrocution were of a light orange-red color. The serums obtained before death were of a normal yellow color. Spectroscopic examination of the serums after electrocution showed the presence of oxyhemoglobin absorption bands. Spreads of the various samples of blood treated with Wright's stain revealed no changes in the morphology of the erythrocytes and no significant changes in the types and respective percentages of leukocytes following electrocution.

From the analyses of the whole blood it may be seen that in each person following electrocution the proportion of cells in the blood and the specific gravity of the blood were increased and that the concentrations of sugar, uric acid, hemoglobin, total solids, chloride and total phosphorus in the blood were increased in all of the samples in which these were determined. The concentration of carbon dioxide of the blood was decreased in subjects T and M. No significant change in

6. Sunderman, F. W.; Austin, J. H., and Camac, J. G.: J. Clin. Investigation **3**:37, 1926. Sunderman, F. W.: ibid. **11**:615, 1931. Sunderman, F. W.; Austin, J. H., and Williams, Priscilla: ibid. **11**:1261, 1932.

the concentrations of urea and creatinine was observed. In subject T the proportion of total base was increased and in M it was decreased following electrocution.

From the analyses of the serum it may be seen that in each person after electrocution the specific gravity and the concentration of total solids and of potassium in the serum were increased and that the concentration of carbon dioxide was decreased. In two of the electrocuted persons the specific conductivity and the concentration of total

TABLE 1.—*Results of Analysis of Whole Blood Before and After Electrocution*

Subject		Cells in Whole Blood, per Cent	Solids, Gm. per 100 Cc.	Hemoglobin, Gm. per 100 Cc.	Total Base, mEq. per Liter	Chloride, mEq. per Liter	Carbon Dioxide, Volume per Cent	Total Phosphorus, Mg. per 100 Cc.	Sugar, Mg. per 100 Cc.	Urea Nitrogen, Mg. per 100 Cc.	Creatinine, Mg. per 100 Cc.	Uric Acid, Mg. per 100 Cc.	Specific Gravity, 20°/20°
W	Before	51.5	.....	16.0	.....	....	..	....	102	14	1.5	3.3	.....
	After	55.0	.....	16.5	.....	....	..	....	128	12	1.6	3.5	.....
T	Before	47.8	20.78	14.8	111.7	80.8	56	36.3	84	11	1.2	3.5	1.0586
	After	55.5	23.37	16.9	115.3	86.6	43	43.5	112	12	1.3	4.6	1.0654
M	Before	45.5	20.43	14.2	115.3	77.0	54	38.3	95	9	1.1	4.0	1.0476
	After	50.0	21.77	14.8	109.0	79.4	39	41.2	218	10	1.2	5.9	1.0612

TABLE 2.—*Results of Analysis of Serum Before and After Electrocution*

Subject		Serum in Whole Blood, per Cent	Solids, Gm. per 100 Cc.	Total Base, mEq. per Liter	Specific Conductivity 20 C. Mhos 10^{-5}	Potassium, mEq. per Liter	Chloride, mEq. per Liter	Carbon Dioxide, Volume per Cent	Inorganic Phosphorus, Mg. per 100 Cc.	Cholesterol, Mg. per 100 Cc.	p_H	Depression of Freezing Point, Degrees C.	Specific Gravity, 20°/20°
W	Before	48.5	9.41	145.8	1172	4.0	98.1	60	4.1	238	7.38	0.522	1.0285
	After	45.0	9.84	144.8	1164	5.1	98.5	58	4.7	277	7.34	0.544	1.0305
T	Before	52.2	8.09	145.1	1243	...	101.8	66	...	...	....	.....	1.0259
	After	44.5	10.08	158.3	1278	6.5	104.8	49	...	...	....	.....	1.0312
M	Before	54.5	8.80	146.0	1199	...	100.2	58	...	...	....	.....	1.0274
	After	50.0	10.08	140.5	1164	7.4	97.1	42	...	...	....	.....	1.0307

base in the serum were decreased; in the third the values for these were increased. The concentration of chloride in the serum showed very little change in subject W; it was increased in subject T and decreased in M. In subject W the concentration of inorganic phosphate and the freezing point of the serum were increased; there was also a slight diminution in the p_H value.

In interpreting the data we have elected to calculate the values of the various components of the blood in relation to the value of hemoglobin, assuming that, of the various components, the hemoglobin in

the blood would have remained unchanged in amount. The results of these calculations are given in table 3. It may be seen that in each subject there was a striking decrease in the amounts of water, total base and chloride in the serum. The loss of chloride in all three subjects was proportional to the loss of water, amounting to approximately 100 milliequivalents of chloride lost per kilogram of water lost. The solids of the serum and of the cells and the protein of the serum remained practically unchanged in amount, but the quantity of cell water per gram of hemoglobin was increased. Whether expressed in relation to hemoglobin or in relation to volume the values for carbon dioxide were decreased and the values for blood sugar were all increased.

Spinal fluid was withdrawn from subject M after electrocution. The fluid was colorless; the concentration of chloride was 124.7 milliequivalents per liter, and the freezing point was depressed by 0.539 C.

TABLE 3.—*Values of Components Calculated Per Gram of Hemoglobin*

Subject		Serum Water, Gm.	Serum Chloride, mEq.	Serum Total Base, mEq.	Serum Solids, Gm.	Cell Water, Gm.	Cell Solids, Gm.	Serum Carbon Dioxide, mM.	Blood Sugar, Mg.
W	Before	2.8	0.30	0.44	0.29	...	...	0.81	6.4
	After	2.5	0.27	0.40	0.28	...	...	0.70	7.8
T	Before	3.3	0.36	0.44	0.29	2.3	1.2	1.01	5.7
	After	2.4	0.28	0.42	0.27	2.4	1.2	0.58	6.6
M	Before	3.6	0.39	0.56	0.35	2.3	1.2	0.90	7.7
	After	3.1	0.33	0.48	0.35	2.5	1.2	0.64	14.7

COMMENT

One of the most significant findings in our analyses is the dehydration of the serum with proportionate loss of chloride. With the exception of the slight gain in water the cells appear to be little affected. It seems unlikely that the dehydration was caused by excessive temperature of the body since the rectal temperature of M secured immediately after withdrawal of the current was only 39 C. (102.2 F.); rather, it was probably the result of passage of water and electrolyte to extravascular areas. There was, moreover, no evidence of coagulation of any of the blood proteins. The only burns which were observed were at the sites of the electrodes. It should be pointed out that the blood obtained from the right side of the heart may not necessarily be the blood there at the moment in which the current was first applied. Physicians attending electrocutions report that occasionally heart sounds are heard after the first contact with the current.

The presence of oxyhemoglobin absorption bands in the serum probably represents either a small amount of hemolysis or a release of muscle

hemoglobin. It has been shown histologically that muscle fibers are ruptured throughout the body during contact with high tension currents. The occurrence of hemolysis or of escape of myohemoglobin is evidenced by the increased values of potassium and phosphorus in the serum following electrocution inasmuch as potassium and phosphorus are of higher concentration in erythrocytes and muscle tissue than in serum. Although no analyses for potassium in the serums of T and M were obtainable before electrocution, the concentration of potassium in the serum of both after electrocution was greatly increased above the normal range.

The increase in the concentration of sugar in the blood may be related to the increasing fear as the condemned person approaches the electric chair. At the time the initial samples were collected T, M and W were outwardly calm. The cause of the diminution in the carbon dioxide is not apparent. The possibility of hyperventilation during the last few minutes is obvious; however, in retrospect no unusual hyperpnea was recollected.

SUMMARY

Blood was obtained from three condemned criminals before and after their electrocution. The analyses indicate a loss of water and its salts from the serum to extravascular areas and a slight gain of water in the cells. We are unable to determine whether the presence of oxyhemoglobin absorption bands in the serum following electrocution was the result of slight hemolysis or of an escape of myohemoglobin into the serum.

TUBULAR TUBERCULOSIS OF THE LIVER

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Tuberculosis of the intrahepatic bile ducts is generally spoken of as tubular tuberculosis of the liver. The development of this type of tuberculosis has been a subject of considerable debate. In this paper we shall present three typical cases.

In the literature of recent years there has been a strong tendency to regard tuberculosis not as a disease limited strictly to one organ but as one in which a generalized spread occurs more often than was formerly suspected. In this generalized spread the liver is infected in a very high percentage of cases. In more than 50 per cent of all autopsies on tuberculous patients we find miliary tubercles in this organ. In cases in which cavernous destruction of the lungs is fatal, this terminal spread is of little importance. In addition to terminal hepatic miliary tuberculosis there are other tuberculous lesions of the liver: (1) miliary tuberculosis as a part of generalized miliary tuberculosis, (2) focal tuberculosis, which is subdivided into nodular forms (single or multiple conglomerate tubercles forming either tumor-like tuberculomas or abscesses) and finally (3) tuberculosis of the bile ducts, the so-called tubular tuberculosis. Morris [1] reported one case of tuberculous cavitations of the liver in 1930 and collected eleven other instances from the literature, only three of this series being reported in the American literature.

In our autopsy service we have encountered three cases of tuberculosis of the bile ducts which show several interesting points.

REPORT OF CASES

CASE 1.—*Clinical Histroy.*—A Filipino aged 20 had come to California in perfect health three years prior to entering the San Francisco Hospital. There was no familial history of tuberculosis. One year before entering the hospital he had a severe pain in the right upper part of the chest, associated with cough, both for one month. He entered the hospital with the complaint of an increasing swelling in the left lower quadrant of the abdomen, loss of weight and night sweats.

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1. Morris, E.: Am. Rev. Tuberc. **22**:585, 1930.

Physical examination showed an undernourished man with a large fluctuant swelling in the left groin. Roentgen examination showed extensive fine mottling of the apexes of both lungs, increased density of the pulmonary roots and tuberculosis of the fourth and fifth lumbar vertebral bodies.

The psoas abscess was drained, and the patient was put in a posterior plaster cast. He was dismissed and later readmitted with an abscess developing in the psoas muscle on the right side. Hibbs' spinal fusion operation was done. In the following year the abscesses continued to drain.

With the development of abdominal pains, particularly in the right upper quadrant, followed by mild jaundice and fever of the septic type, the patient died.

Necropsy.—The essential observations were extreme emaciation, tuberculosis of the spine with abscesses of both psoas muscles, miliary tuberculosis of the lungs, equally distributed and without cavitation, and miliary tuberculosis of the spleen, kidney and adrenal glands. The most remarkable lesions occurred in the liver.

The liver barely reached the costal margin and xiphoid process, weighed 1,200 Gm., and measured 20 by 14 by 6 cm. The capsule was smooth and purple. The cut section revealed multiple and discrete abscess cavities filled with greenish brown granular material. The necrotic material washed out easily, leaving a ragged capsule 2 mm. in thickness. The abscesses were of various sizes, the largest measuring 2.5 cm. in diameter (fig. 1). The extrahepatic bile ducts and the gallbladder were normal and contained thick greenish black bile.

In spite of careful search, no ulceration was found in the small or large bowel, the mucosa being perfectly smooth. Over the posterior surface of the cecum were two rounded yellowish nodules, the largest 1 cm. and the smallest 0.5 cm. in diameter. These were elevated above the peritoneum approximately 3 mm. There were a few enlarged pericecal mesenteric lymph nodes. The peritoneal surfaces of the lymph nodes were smooth, and cut sections showed the nodes to contain dry cheesy material.

Histologic Examination.—The gross and microscopic examination failed to reveal any primary lesion in the lungs or peribronchial lymph nodes. The lungs showed only a fibrohyaline thickening of the apical pleura.

Sections of a lymph node attached to the right side of the diaphragm showed a centrally caseated lesion, with a heavy connective tissue capsule.

The liver showed mild passive congestion with cloudy swelling of the liver cells. Scattered throughout the parenchyma, especially in the areas of periportal connective tissue, were many miliary epithelioid cell tubercles with central giant cells. In the section were three abscesses filled with a brown-stained caseous material containing rests of cuboidal epithelial cells from destroyed bile ducts. About each abscess was a moderately thick fibrous connective tissue capsule. The inner surface was formed by typical tuberculous granulation tissue. In another place there was a longitudinal section of a medium-sized bile duct, surrounded by irregularly arranged epithelioid and giant cells. The epithelium of this duct was well preserved on one side, while on the opposite side the epithelium was desquamated into the lumen by a tubercle rupturing into the bile duct from the outside (fig. 2).

A section of the subserosal encapsulated nodule from the cecum showed it to be composed of a reticulum of loose connective tissue including a caseous necrotic center and surrounded by a very dense connective tissue capsule at the periphery. Around the periphery of the caseated area were epithelioid cells, many lymphocytes

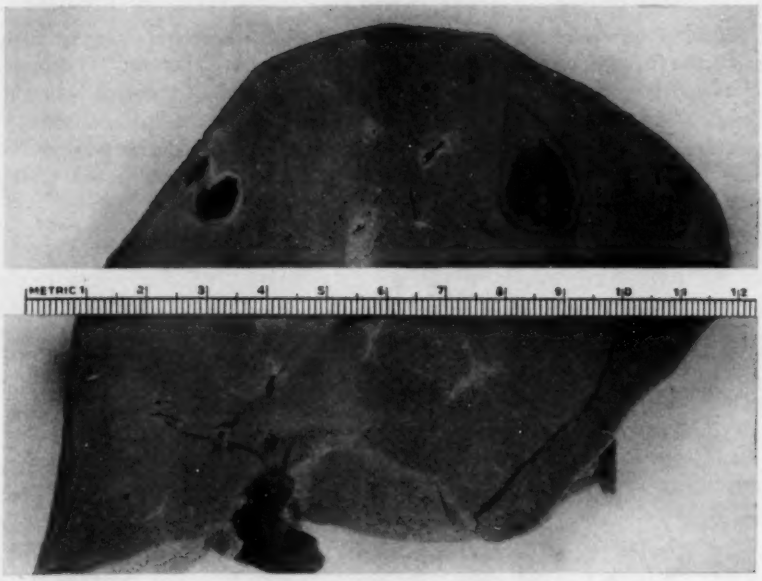

Fig. 1 (case 1).—Cut surface of the liver showing tuberculous abscesses around bile ducts.

Fig. 2 (case 1).—Longitudinal section showing tubercles breaking into the lumen of a bile duct about which they have developed; × 50.

and dilated blood vessels but no giant cells or typical tubercles. The musculature and mucosa of the cecum were normal. The whole formation resembled an encapsulated primary lesion in the early stage of consolidation (fig. 3).

Bacteriologic Examination.—The lungs showed myriads of tubercle bacilli, as did smears from the sinus tract of the abscess in one of the psoas muscles.

The abscesses of the liver also contained numerous brownish red acid-fast bacilli.

In this case the clinical course and the morbid anatomy revealed that the man died of an advanced tuberculosis of the spine, with a miliary spread into the inner organs complicated by tuberculous abscesses along the intrahepatic bile ducts.

Case 2.—*Clinical History.*—A Filipino aged 25 entered the San Francisco Hospital with the complaint of pain in his back for three months.

Physical examination showed a gibbus of the upper lumbar vertebrae.

Hibbs' operation was performed, which was followed by continuous fever, the temperature rising to 103 and 104 F., progressive emaciation and after two weeks death in convulsions.

Fig. 3 (case 1).—A subserosal tuberculoma of the cecum; × 5.

Necropsy.—At autopsy tuberculosis of the ribs and of the vertebral bodies was found. Both lungs showed a moderate miliary spread; no calcifications and no foci in the bronchial lymph nodes could be demonstrated. A large ulceration was present in the ileum, proximal to the ileocecal valve. The ulcer was surrounded by a number of white punched-out ulcers in the cecum. The regional lymph nodes were enlarged and showed caseous areas on cut sections.

The liver showed mild atrophy, measuring 26 by 17.5 by 6.5 cm. The capsule was smooth. In cut sections many small white nodules were seen, which seemed to be many bile cysts surrounded by fibrous tissue and containing greenish necrotic material.

Histologic Examination.—Microscopically the liver showed numerous tubercles in the periportal connective tissue, some of them large, extensively caseated and causing destruction of the walls of the bile ducts.

This case also is one of a young Filipino native in whom, without signs of pulmonary infection, tuberculosis of the bone developed which was complicated by tuberculosis of bile ducts and of the liver. The site of the primary infection must be assumed to have been in the intestine.

CASE 3.—*Clinical History.*—An American salesgirl aged 24 entered the San Francisco Hospital with the complaint of cough for a year and slight hemoptysis six months previously. She had a brother who five years previously had been discharged from the army with pulmonary tuberculosis. Four years prior to entry she had pleurisy of the left side, followed by a "nervous breakdown" and fever.

At entry the motion of the left side of the chest was restricted, and the left side of the diaphragm did not move. Percussion was dull over the left lung, with bronchial and amphoric breath sounds as well as many moist râles on auscultation. Many crepitant râles were present over the right apex after coughing. The heart and extremities were normal, the reflexes were slightly overactive, and secondary anemia was present. Roentgen examination of the chest revealed the left lung riddled with cavities and the right lung mottled with recent tuberculous infiltrations.

Artificial pneumothorax was impossible because of dense adhesions.

The patient became progressively worse, complained of pain in the right upper quadrant of the abdomen, and died three months after entry.

Necropsy.—The observations confirmed the clinical diagnosis of pulmonary tuberculosis with cavitation. There was gross tuberculosis of the terminal part of the ileum and of the cecum. All the mesenteric lymph nodes were enlarged and had undergone caseous necrosis. The peribronchial lymph nodes were caseous but showed no calcification.

The liver had a smooth light brownish purple capsule, was markedly shrunken, weighed 1,000 Gm., and measured 24 by 15 by 6 cm. On the cut section one abscess, approximately 8 mm. in diameter, of the previously described tubular type, was seen along a bile duct. The central caseous material filling the abscess was stained greenish brown with bile.

The other organs showed no gross tuberculosis.

Histologic Examination.—Sections of the lungs and intestines showed typical tuberculous lesions with many epithelioid cells, a few giant cells and extensive central caseation.

The liver showed a rather marked passive congestion; many of the bile capillaries were distended and plugged with bile. Throughout the parenchyma were a few epithelioid tubercles with Langhans' giant cells. In one area a large "abscess" occurred which was filled with bile-stained caseous material. Numerous epithelioid cells were present at the margin, and a marked fibrous connective tissue capsule, infiltrated with round cells, surrounded the area. A large bile duct at the periphery of the abscess showed a tubercle extending into its lumen; the epithelium of the affected side was desquamated into the lumen, mixed with inflammatory cells, whereas the epithelium of the opposite side was intact (fig. 4).

All the pericecal lymph nodes showed most of the lymphatic tissue replaced by caseous necrosis surrounded by specific granulation tissue.

Bacteriologic Examination.—Myriads of tubercle bacilli were found in the lungs, and in the caseous mesenteric lymph nodes occasional acid-fast bacilli were seen.

This case was one of typical progressive pulmonary and extensive intestinal tuberculosis with caseation of the mesenteric, peribronchial and periportal lymph nodes, complicated by a secondary tuberculous cholangeitis with abscess formation.

MODES OF INFECTION IN TUBERCULOSIS OF THE BILE DUCTS

The modes of infection in tuberculosis of the bile ducts have often been under discussion. In disseminated miliary tuberculosis originating from the rupture of a tubercle into the pulmonary vein the bacilli invade the liver through the hepatic arteries. Kotlar and Lichtenstein [2] expressed the opinion that the bacteria lodge at the point of branching of the capillaries. Pagel [3] supported this idea by showing a giant cell in a tubercle in the liver which had apparently arisen in the wall of an arterial capillary in the periportal tissue.

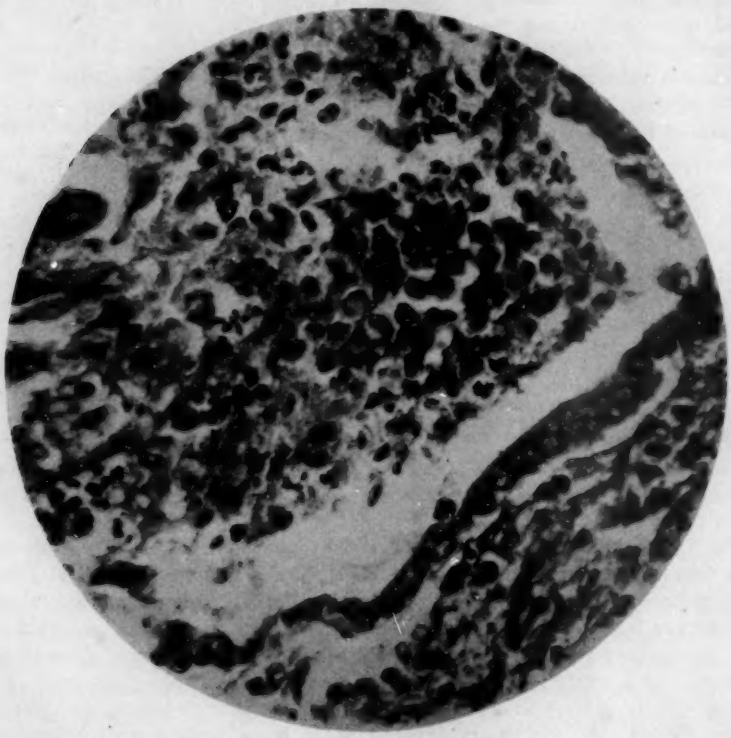

Fig. 4 (case 3).—Cross-section showing extension of a tubercle into the lumen of a bile duct about which it has developed; × 450.

The more widely disseminated miliary and submiliary tubercles of the liver found in association with tuberculosis of the intestines are thought to originate from thromboses in the capillaries of the portal system. Strauss,[4] confirming this assumption, succeeded in obtaining

2. Kotlar and Lichtenstein, cited by Joest and Ziegler.[9]

3. Pagel, W.: Die allgemeinen pathomorphologischen Grundlagen der Tuberculose, Berlin, Julius Springer, 1927.

4. Strauss, cited by Pagel.[3]

positive results in animals on injection of portal blood from patients who died of intestinal tuberculosis.

These two types of miliary tuberculosis show no tendency toward cavity formation or toward growth of the lesion by continuity or confluence to larger nodules.

For the development of true tuberculous abscesses Kaufmann,[5] Sheldon[6] and lately Morris[1] emphasized the lymphatics as probable conveyers of infection starting from tuberculous peritonitis.

For the infection of the bile duct there is also a fourth possibility, which was considered many years ago by Simmonds,[7] who thought that the "infectious agent" from the intestines might ascend through the bile ducts and then infect the mucosa. As a more probable way, however, Simmonds described the same development which was found in our slides, namely, tubercles rupturing from the outside into the bile ducts, proposing the name "tuberculous pericholangeitis," analogous to "peribronchitis."

Finally, Huebschmann[8] emphasized the possibility that tubercle bacilli may be excreted through the liver as through the kidney or that, instead, they may get into the bile capillaries by leakage from the blood capillaries. Following this he described a case of primary tuberculous cholangeitis of catarrhal character. Attempting to confirm his findings, we tried acid-fast strains on numerous sections of liver and were able to find tubercle bacilli only in the centers of the caseous areas, none being found in bile capillaries or bile ducts which anatomically were free from tuberculous changes.

On the other hand, it was proved by Fraenkel and Krause,[2] Joest and Ziegler[9] and Calmette and Guerin[10] that bile of tuberculous animals and men gives positive results up to 70 per cent when inoculated into guinea-pigs, even when there is no gross tuberculosis of the liver. A more careful investigation of these livers by Kotlar and Lichtenstein,[3] however, revealed again the picture of intrahepatic tuberculous cholangeitis. Medlar and Sasano[11] reviewed the whole problem in 1924 and came to the same conclusion. They believed that in guinea-pigs small

5. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, Berlin, W. de Gruyter & Company, 1922.

6. Sheldon, W. P. H.: Lancet **2**:1343, 1927.

7. Simmonds, M.: Deutsches Arch. f. klin. Med. **27**:448, 1880.

8. Huebschmann, P.: Pathologische Anatomie der Tuberculose, Berlin, Julius Springer, 1928.

9. Joest and Ziegler: Verhandl. d. deutsch. path. Gesellsch. **16**:178, 1913.

10. Calmette and Guerin: Compt. rend. Acad. d. sc. **148**:601, 1909.

11. Medlar, E. M., and Sasano, K. T.: Am. Rev. Tuberc. **10**:351, 1924.

infarcts often precede true tuberculous destruction and invasion of the bile ducts. They concluded that "tubercle bacilli in the bile mean tuberculosis of the liver."

Therefore, we are confronted with the query as to why miliary tuberculosis of the liver is seen so often, in contrast to tuberculosis of the bile ducts, which is extremely rare. Sasano and Medlar [12] found that the hepatic tuberculosis of the nonimmunized guinea-pig is of the bile duct type. It is possible that not only may the number of tubercle bacilli be important for the development of the "abscesses," but also the allergic state of the whole organism. The development of tubular, bile duct tuberculosis might be due to the fact that the patients are in the primary stage of tuberculous infection and as yet possess relatively low resistance.

RÔLE OF ALLERGY AND IMMUNITY IN THE DEVELOPMENT OF BILE DUCT TUBERCULOSIS

According to Ranke,[13] tuberculosis may be divided into the periods of primary and secondary infection. The former is generally localized in the lungs, less often in the intestines, and heals by calcification or ossification of the focus in the lung and in the peribronchial lymph nodes. The latter or second period of the common pulmonary tuberculosis is usually due to reinfection and is strictly limited to the lung. Recent research has made it obvious that a period of generalized dissemination by way of the lymph and blood streams often occurs between these two main periods. This condition of generalized dissemination is found in children and also in young adults who come in contact with tuberculosis very late. Borrell's [14] autopsies of tuberculous Negroes of the colonial armies in France during the World War showed this type of rapidly progressive pulmonary consumption with caseation of all the lymph nodes. In Negroes, who on arrival in France gave a positive tuberculin reaction in only 5 per cent of the cases, the relation between mortality and morbidity was 1:2; in the white troops it was 1:17. In autopsies of Senegal Negroes Gruber [15] had the same experience, finding fresh caseation of all lymph nodes. He also mentioned the frequent occurrence of bile-stained abscess cavities in the liver.

Opie [16] found similar conditions among Negroes in the southern states and in Jamaica. He found the death rate from tuberculosis four

12. Sasano, K. T., and Medlar, E. M.: Am. Rev. Tuberc. **10**:392, 1924.

13. Ranke, K. E.: Ausgewählte Schriften zur Tuberkulose-pathologie, Berlin, Julius Springer, 1928.

14. Borrell: Ann. Inst. Pasteur **34**:105, 1920.

15. Gruber, G. G.: Ztschr. f. Tuberk. **33**:23, 1920.

16. Opie, E. L.: Am. Rev. Tuberc. **22**:603 and 613, 1930.

times higher in colored people than in the white population. In the Negroes tuberculosis usually ran a rapidly fatal course, indicating low resistance.

Morris [1] mentioned a private communication from Fucar in which it is stated that tuberculosis of the liver is more common in races not possessing natural immunity against tuberculosis. The fact that in the first two of our cases severe generalized tuberculosis with abscesses of the liver developed in natives of the Philippines who had been perfectly healthy before they came to California points in the same direction.

SITE OF THE PRIMARY FOCUS AND ITS RÔLE IN THE DEVELOPMENT OF BILE DUCT TUBERCULOSIS

Another factor is the site of the primary focus. The intestinal primary infection does not necessarily leave any scar in the intestine itself, because the tubercle bacillus may pass through the normal mucosa, manifesting itself only in the regional lymph node, whence it may be conveyed to the blood stream. Calmette in his protective feeding with BCG made use of this condition. In Germany, owing to an unfortunate mistake, in which virulent and nonvirulent cultures were changed, seventy-two children died of tuberculosis due to ingestion, and were examined post mortem by Schuermann.[17] In 98 per cent of the cases he found the primary infection in the small bowel with a hematogenous spread throughout the entire body. Whereas these children died very early from meningitis, only miliary tuberculosis was found in the internal organ. No large caseous foci were found.

Ranke [18] described tuberculosis of bone occurring in two children in whom at autopsy he could detect no primary lesion in the lungs. Consequently, he emphasized that early generalized dissemination is quite common in cases of extrapulmonary tuberculous primary infection.

The anatomic picture of the primary intestinal lesion, according to Schuermann,[17] consisted, in 100 per cent of his cases, of a typical tuberculous primary infection in each of two structures, i. e., a tuberculous ulcer in the intestine and caseation of the regional lymph nodes. Ulcers were not found beyond the ileocecal valve.

Siegmund [18] described the fate of intestinal primary infection as being very similar to that of the primary infection in the lung. He mentioned that foci are often found in the submucosal, muscular and

17. Schuermann, P.: Beitr. z. Klin. d. Tuberk. **81**:294, 1932.

18. Siegmund, H., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 4, pt. 3, p. 371.

subserosal layers, the latter being transformed into a vascular nonspecific granulation tissue. In this, a tendency toward fibrous transformation is evident, and sometimes the latter process surrounds an old area of caseation. Schuermann [17] found that calcification starts early in children, even fifty-eight days after infection. In adults, however, Rosenkranz [19] noted that the primary focus is more apt to remain in a stage of soft, incomplete calcification instead of progressing to ossification.

To investigate the infectious potency of calcified nodes, Weber,[20] Rabinowitsch,[21] Anders [22] and Opie and Aronson [23] inoculated mesenteric lymph nodes in guinea-pigs and obtained from 26 to 100 per cent positive results, thus confirming the observation that even calcified lymph nodes in a high percentage of cases contain virulent bacilli.

COMMENT

In our first case a guinea-pig inoculation test was not made with nodules from the cecum, but the histologic picture of the well encapsulated, centrally caseous tuberculomas in the subserosa speaks strongly in favor of their being a part of the primary tuberculous lesion, especially so in the absence of any recent tuberculous ulceration in this part of the bowel and the absence of any sign of generalized peritonitis. The presence of one caseous nodule on the diaphragm in the line of the lymph current from the abdomen upward is also confirmatory of this assumption. In neither the second nor the third case did we find an old calcified focus. Extensive caseation was present in the mesenteric lymph nodes in the second case and in the mesenteric and peribronchial lymph nodes in the third case. This is compatible with the typical picture of early generalized dissemination immediately following the primary infection.

In the third case the site of the primary infection could not be ascertained with certainty, but the caseation of the lymph nodes from the mesenteric up to the cervical level suggests an intestinal primary infection.

Siegmund [18] described a case and stated that an intestinal primary lesion that starts with an ulceration of the mucosa may continue to grow, thus becoming a chronic tuberculous ulcer. Thus, in cases in which no primary focus can be identified with certainty but in which many caseous changes are present in the lymphatics, it may finally become impossible to decide whether the ulcer is secondary to the

19. Rosenkranz, K.: Beitr. z. Klin. d. Tuberk. **85**:584, 1934.
20. Weber, cited by Anders.[22]
21. Rabinowitsch, cited by Siegmund.[18]
22. Anders, K.: Beitr. z. Klin. d. Tuberk. **81**:260, 1932.
23. Opie, E. L., and Aronson, J. D.: Nat. Tuberc. A. Tr. **23**:265, 1927.

pulmonary tuberculosis or whether the pulmonary tuberculosis is secondary to a progressive intestinal primary infection.

If we review in brief the different theories which have been published on the origin of caseous cholangeitis in man, we find that Lotheissen [24] in 1912 made a survey of forty-seven cases of conglomerate tubercles and tuberculous "abscesses," and that in only five of these, according to Roessle,[25] were the tubercles in connection with the biliary system. Concerning the etiology of tuberculosis of the bile ducts, Lotheissen agrees with Pertik [26] that the infection originates by way of the blood stream; namely, tubercle bacilli lodge in the periportal vessels and establish a focus which later ruptures into the bile ducts. Bagozzi,[27] who made a second extensive survey of this subject in 1931, collected nineteen new cases occurring since 1912 and added three of his own. From this series he confirmed the previous statements of Simmonds [7] and Lotheissen [24] that tuberculosis of the liver may be found at any age. He put more importance on the finding of caseous lesions in the mesenteric and periportal lymph nodes and believed that the bacilli travel along the lymph current of the hepatic hilus into the liver, starting from a hematogenous infection of the appendix. Although he offered this theory mainly for the cases of tuberculous abscesses and conglomerate tubercles, it seems to be unnecessarily complicated to assume that the blood stream infection might locate itself first in the appendix and then later enter the lymphatics. Morris [1] in the latest survey in the English literature followed Sheldon [6] and Bagozzi [27] in this theory but assumed that the hepatic focal infection may also originate from tuberculous peritonitis, following the lymph vessels of Glisson's capsule. The possibility that the bacilli invade the extrahepatic bile ducts and then ascend, as Gilbert and Claude [28] found in producing tuberculous cholangeitis in animals, is rejected by all authors.

Today, we believe that the mode of invasion is not as significant as the allergic condition of the organism which permits caseation of tubercles in the liver. In cases of intestinal primary infection, especially, the bacilli might invade the liver in both ways, viz., by way of the lymphatic drainage and by way of the blood stream from the portal system, thus overloading it with toxins and bacilli.

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24. Lotheissen, G.: Beitr. z. klin. Chir. **81**:1, 1912.

25. Roessle, R., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 5, pt. 1, p. 270.

26. Pertik: Ergebn. d. allg. Path. u. path. Anat. **8**:279, 1904.

27. Bagozzi, U. C.: Arch. ital. di anat. e istol. pat. **2**:813, 1931.

28. Gilbert and Claude, cited by Lotheissen.[24]

CONCLUSIONS

The anatomic findings in our cases and the various pathogenetic considerations indicate that two factors must be necessary in the origin and extension of tuberculous cholangeitis. The first is an early lymphatic and hematogenous generalized spread of the primary infection before resistance has been established. The second is an intestinal site for the primary infection and extensive caseation of the mesenteric glands, both of which seem especially to favor the formation of caseous tubercles in the periportal tissue, in contrast to the miliary cellular tubercles commonly found in the end phase of chronic pulmonary tuberculosis with secondary intestinal tuberculosis or a terminal miliary spread. The bile ducts themselves are not primarily infected, but the periportal tubercles rupture into their walls.

SUMMARY

Three cases of tuberculosis of the intrahepatic bile ducts with "abscess formation" are described. The microscopic picture of the liver in these cases shows a rupture of caseous tubercles into the bile ducts from the periportal tissue. The excretion of virulent tubercle bacilli with the bile is always due to microscopic ruptures of this kind. Development of tubular tuberculosis of the liver is favored by an early generalized spread of the primary infection, especially when the latter occurs in the intestine. This type of tuberculosis is usually found in young children and in races that do not have high resistance against infection by Koch's bacillus. Possible ways of infection are discussed. Intestinal primary infection tends to spread easily and to retain virulent bacilli for a long time.

GENESIS OF INTESTINAL INFARCTION FOLLOWING EMBOLIZATION OF THE SUPERIOR MESENTERIC ARTERY

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BROOKLYN

Abundant evidence from autopsies has established the fact that embolic occlusion of the superior mesenteric artery results in a hemorrhagic infarct of the small intestine. One would expect, however, that an occlusion of the artery would result in anemic necrosis of the small intestine rather than in a hemorrhagic infarct. The explanations given in the literature are many and various. Especially striking is the fact that experimental ligation of the superior mesenteric artery has led to contradictory results in the hands of different observers. Some (Litten,[1] Madelung,[2] Sprengel[3]) found hemorrhagic necrosis; a smaller group (Niederstein,[4] Beckmann[5]) found anemic necrosis, while still others (Ravenna,[6] Bolognesi[7]) found both anemic and hemorrhagic necrosis, as well as some atrophy of the intestine. In the following experiments the mesentery and the intestine of the living animal were observed microscopically with the hope that such direct observation would clarify the mechanism of the result of ligation and also that of the collateral circulation; for these purposes the intestine seemed especially suited.

EXPERIMENTS

The experiments were performed on rats. To facilitate the macroscopic observation of the relatively short mesentery of the rat, a simple apparatus was constructed as follows:

A hole was drilled into one end of a board approximately 35 by 12 cm., and the latter was mounted on a tripod by a movable support. A right-angled notch about 4 cm. wide and from 3 to 4 cm. deep was made in the front edge of the board. On each end of this notch was nailed a small block of wood, 2 by 2.5 cm. Across these blocks, a broad glass slide (broader than the ordinary slide, which is too narrow) was placed, and was held in place by a rubber band, from below. In order to maintain the mesentery and the rat in the proper position, a

From the Department of Pathology, Crown Heights Hospital.

1. Litten, M.: Virchows Arch. f. path. Anat. **63**:329, 1875; Deutsche med. Wchnschr. **15**:145, 1889.
2. Madelung: Arch. f. klin. Chir. **27**:277, 1882.
3. Sprengel: Arch. f. klin. Chir. **67**:587, 1902.
4. Niederstein: Deutsche Ztschr. f. Chir. **85**:710, 1906.
5. Beckmann, Otto: Virchows Arch. f. path. Anat. **13**:501, 1858.
6. Ravenna, Ettore: Riv. Veneta di sc. med. **50**:136, 1909.
7. Bolognesi, Giuseppe: Virchows Arch. f. path. Anat. **203**:213, 1901.

flexible wire netting was used, which was rolled up in such a manner that it kept the rat in a loose but sufficiently secure position. On each side, between the glass plate and the wooden blocks, a layer of cotton wool was inserted; this covered the parts of the intestine which were not in use. The mesentery and the intestine were irrigated through the cotton padding with physiologic solution of sodium chloride at body temperature; a drop by drop flow was sufficient. The stage was screwed off an ordinary microscope, and the condenser was placed in the notch. A Leitz no. 2 objective and a no. 1 or 3 ocular gave a magnification of from 30 to 50, which sufficed for the purposes in view (fig. 1).

Ligation of the Superior Mesenteric Artery.—The artery was easily found on the left side near the left kidney after the intestines were displaced over the opposite side.

The small intestine blanched immediately and after a few minutes of hyperperistalsis remained strongly contracted. The macroscopically visible vessels,

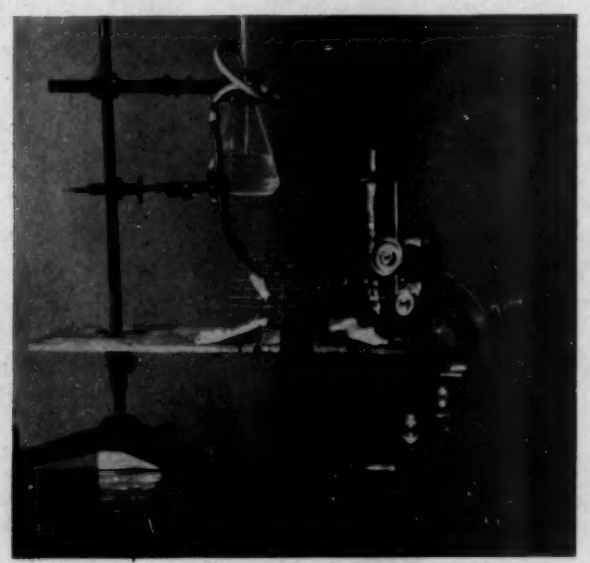

Fig. 1.—An apparatus for the microscopic study of the mesentery of the rat. The lamp is omitted. The microscope, with its stage removed, fits into the notch in front, which is bridged over by the glass slide.

both arteries and veins, were narrower than normal, although distinctly visible as red streaks. The mesentery became shortened, and the intestine tended to be drawn into the abdominal cavity. Microscopically, in the living animal, one saw empty vessels and capillaries appearing as white threads. In isolated areas of the mesentery immediately or shortly after ligation there was some flow in some peripheral vessels, both in a small artery and the corresponding vein or veins. In a few areas of the mesentery there were petechiae. The serosal vessels of the intestine were empty or showed alternating groups of clumped red blood cells and empty spaces.

Two animals died directly after the procedure. The other rats died in from one to two hours or several hours or were found dead the next morning. At necropsy the intestines of the animals that died after several hours were found to be necrotic, softened, friable and usually pale, but sometimes pink owing to

imbibition. Here and there were a few small hemorrhages. Animals that died within from one to two hours showed no changes other than those observed during life, i. e., no necrosis. The lumen of the intestine was dilated little, if at all, and was either empty or filled with a small amount of mucoid or bloody fluid.

Obstruction of the Portal Vein; Ligation of the Vein.—If the ligature was merely slipped around the portal vein and then held taut without actual ligation, the flow of blood in all the visible mesenteric vessels (arteries, veins and capillaries) ceased immediately without any change in the volume of the vessels. These experiments made feasible a direct microscopic study of the gut and mesentery at the instant of occlusion of the vessel.

In the following experiments observation was delayed for the few minutes that elapsed between the ligation of the vessel and the arrangement of the animal on the table.

Immediately after ligation of the vein, and for about one quarter of an hour afterward, the intestine became increasingly redder. After about ten minutes the lumen became definitely wider (fig. 2), and the gut was filled with a bloody mucoid fluid. The mesentery increased in length as the intestine dilated and was easily drawn out. The mesenteric veins were dark red, distended and markedly tortuous. The arteries were relatively narrow and only slightly tortuous. Microscopically, in the living animal, the veins were distended and filled with columns of dark red blood. Some of the small arteries were empty and narrow, others were filled with blood, but there was no flow of blood.

In certain isolated fields there was a current of blood in the small arterioles, veins and capillaries. In general, this was found in any smaller artery that led directly into a vein in a straight line; the smaller branches and small arterioles and venules showed no circulation (fig. 2). Wherever there was no flow in a vein, the corresponding artery was empty, and vice versa. Where flow existed, it was very slow; for instance, in the veins, the red blood cells became clumped in the transit from artery to vein via capillaries. In the arteries, the leukocytes were increased in number; in the veins, groups of leukocytes or free spaces filled with serum were visible between the groups of red blood cells. Numerous petechiae appeared about five to ten minutes after ligation. At about one-half to two hours the macroscopic and the microscopic picture were unchanged except that the reddening had increased in intensity; macroscopically, the columns of blood in the veins were darker than before, but still there was no movement.

All animals died in from two to six hours after ligation. At necropsy the intestine was red, dilated and filled with bloody mucoid fluid; there were numerous hemorrhages, particularly at the root of the mesentery.

Since the mesentery of a normal rat has few vessels, a laparotomy was first performed in which the mesentery was examined microscopically in vivo without ligation of any vessel; fourteen days later another laparotomy was performed. In such previously laparotomized animals the mesentery has many more capillaries and is more easily studied. In six such animals both the superior mesenteric artery and the hepatic artery were ligated. The effect on the intestines as far as anemia and contraction were concerned was apparently greater; otherwise it was similar to that shown in figure 2*A*. *C* and *D* in figure 2 are drawings made from animals of this sort.

COMMENT

The experimental ligation of the superior mesenteric artery on the one hand and of the portal vein on the other yielded unequivocal although dissimilar results. Provided the animal lived long enough

(a few hours) ligation of the superior mesenteric artery produced a simple, so-called anemic necrosis. The gut became softened. A certain amount of imbibition and slight reddening as well as scattered small hemorrhages were visible, to be sure, and microscopically the gut was seen to abound in red, congested capillaries, but there was certainly no indication of a hemorrhagic infarct. On the other hand, after occlusion of the venous tracts the intestine was dark red and suffused with

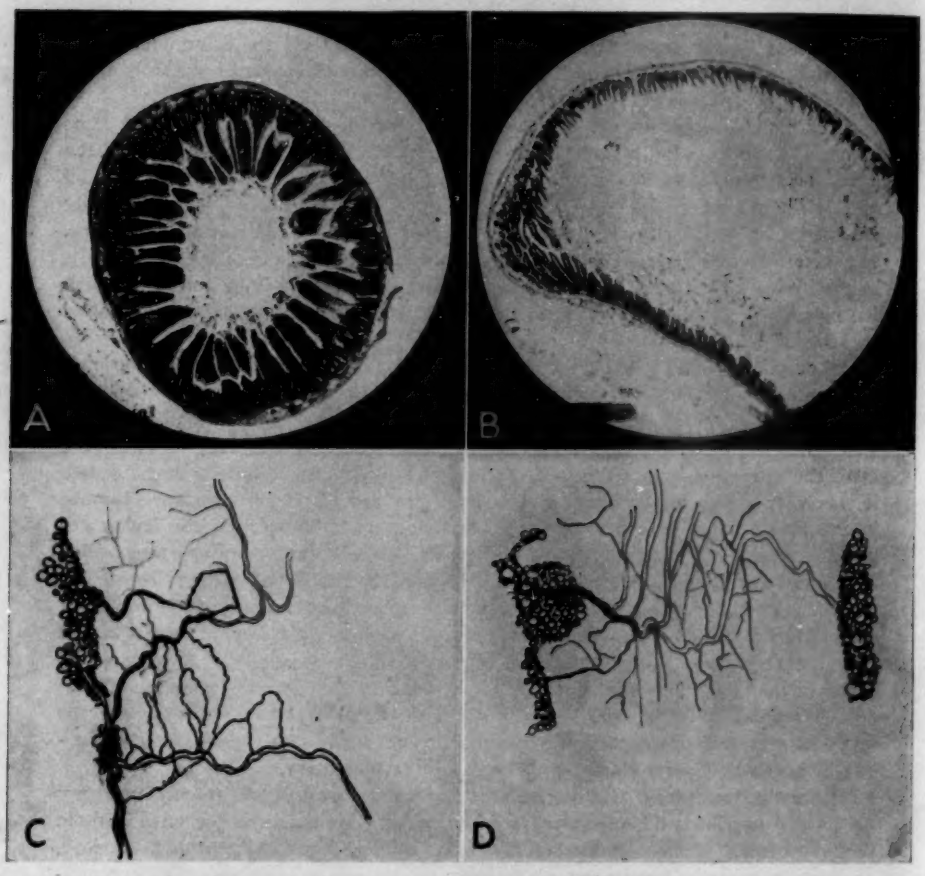

Fig. 2.—*A*, intestine one hour after ligation of the superior mesenteric artery; *B*, intestine one hour after ligation of the portal vein. The lumen in *A* is narrowed; in *B* it is dilated to almost twice the normal size. The same magnification was used for both *A* and *B*. *C*, drawing of a field in the mesentery after ligation of the portal vein; *D*, drawing of a similar field after ligation of the superior mesenteric artery. The black streak on the left side in each field represents the remaining patent blood vessel. All the smaller vessels as well as most of the other fields show complete stasis. The main mesenteric vessels are on the sides, surrounded by adipose tissue.

numerous hemorrhages. With arterial occlusion the lumen of the gut was diminished in size and was essentially empty; after venous occlusion the gut was dilated and filled with bloody mucoid material. These experiments provide corroboration of the findings of those authors who were unable to produce infarction of the gut after ligation of the artery in rabbits (Beckmann [5]) and in dogs (Niederstein,[4] Bolognesi [7]). The experiments, however, are contradictory to the well known findings in man (Klein,[8] Larson,[9] Douglas,[10] Silhol,[11] Moiroud,[12] Lévy-Weissmann,[13] Haberer [14]) and to the experimental results of Litten,[1] Madelung,[2] Ravenna [6] and Rydigier.[15] As far as the experimental results of occlusion of the artery are concerned, the contradiction can be explained. In all reports it is stated that after arterial ligation the intestine is friable, gelatinous and pale, or pink with focal hemorrhages. In addition, Litten often loosened the ligature, doubtless because the reddening and the extension of the hemorrhages did not correspond to the result he expected. Such slight reddening and focal extravasation of blood are, however, present in every necrotic area. Every anemic infarct is red in the first few hours; here one deals with stasis in the capillaries and veins. Microscopically, in the living animal, I was able to observe that such capillaries and veins become dark red because of anoxemia, without any increase in the total quantity of blood present. A similar process takes place, for example, in an extremity after embolization of the aorta or the femoral artery with complete obstruction to the flow of blood (Marcus [16]). In other words, the difference in the experimental results is not in the facts but in the nomenclature. The findings cannot truthfully be described as hemorrhagic infarcts.

On the other hand, when the venous return from the gut is suddenly impeded, marked hyperemia and profuse hemorrhages result. Such results follow ligation of the portal vein. The occlusion results in dark red discoloration, massive hemorrhages and a bloody, increased intestinal content. The result of experimental impedence to the venous return from the intestine, while still not exactly identical with the result of venous thrombosis or of arterial embolism observed in man, is far more analogous to a hemorrhagic infarct than the picture produced by arterial ligation.

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8. Klein, Eugene: Surg., Gynec. & Obst. **38**:385, 1921.
9. Larson, Laurence: Surg., Gynec. & Obst. **53**:54, 1931.
10. Douglas, John: Tr. Am. S. A. **53**:154, 1935.
11. Silhol, M.: Arch. de franco-belges de chir. **27**:1016, 1924.
12. Moiroud, Pierre: Bull. et. mém. Soc. anat. de Paris **94**:441, 1924.
13. Lévy-Weissmann, Marcel: Gaz. d. hôp. **97**:1125, 1924.
14. von Haberer, Hans: Arch. f. klin. Chir. **92**:265, 1910.
15. Rydigier: Berl. klin. Wchnschr. **18**:633, 1881.
16. Marcus, Max: Deutsche Ztschr. f. Chir. **224**:186, 1930.

From this one may conclude that a sudden marked increase of venous pressure is indispensable for the production of a hemorrhagic infarct. Microscopic observation in vivo demonstrates that the hemorrhages are present immediately or in the first few minutes after venous ligation, and that the striking gross hyperemia arises from passively congested capillaries and veins without visible flow of blood. The infarction of any region (in this case the intestine and mesentery) must accordingly be explained on the vascular status of the area involved.

It seems fundamental that there is a difference between the experimental results and the findings observed clinically. The identification of experimental results with the findings in patients at autopsy is no doubt the chief reason why the explanation of the infarction has so far been unsatisfactory. The actual difference between the two is that in man real hemorrhagic infarction of the gut follows embolization of the superior mesenteric artery. In man an embolus arises, in general, from a thrombus in the heart. The existence of cardiac thrombi, however, implies a more or less pronounced condition of stasis and therefore of venous hyperemia; i. e., the condition of the vessels before embolization in man is basically different from that in experimental animals. If in a congested region there is heightened venous and lowered arterial pressure, as occurs after ligation of the portal vein, the tendency toward hemorrhage and infarction comes to the fore. The heightened venous pressure and the increased permeability of the capillaries before embolization constitute the conditions which cause embolization to produce, not a simple necrosis, but a hemorrhagic infarct. It is the same process as in the case of hemorrhagic pulmonary infarction. Here, also, the infarction occurs only when there is stasis. The cause of infarction is not merely embolization but embolization plus venous stasis.

Microscopic observation of the mesentery after ligation of the superior mesenteric artery and after ligation of the portal vein demonstrates that in both cases a certain collateral circulation exists and is visible immediately after ligation. This collateral circulation is more extensive after ligation of the portal vein. Collateral channels that are anatomically demonstrable are known to exist between the superior mesenteric artery, the pancreaticoduodenal artery and the inferior mesenteric artery (Hasse and Grosser, after Litten[1]). The portal vein has anatomically demonstrable collateral communications with the lumbar veins, the hemorrhoidal venous plexus and the coronary and esophageal veins (Anten[17]). In addition, there exists in both cases a collateral circulation in the submucosa of the intestine.

17. Anten, August: Deutsche Ztschr. f. Chir. **224**:53, 1930.

In both cases, however, the collateral circulation is either totally or almost totally inadequate. It does not prevent necrosis, infarction or the death of the animal or of the human being. Microscopic observation shows that the collateral paths are extraordinarily few and completely unconcerned with the process of infarction. Both streambeds, both the larger, which is occluded, and the smaller, which is patent, course side by side. There is no flow in the area observed to be infarcted macroscopically. In the intestine itself there is no visible current; if any exists, it must be as insignificant as in the mesentery. No doubt the flow in isolated arteries and veins which I observed here and there was due to collateral circulation, since the ligature was certainly complete.

It is noteworthy that ligation of the artery or vein results in immediate arrest of the whole circulation except in the few collateral channels. Further, wherever an artery is found patent, there is a corresponding patent vein, and vice versa. When the portal vein is ligated, the flow in the arteries ceases too, and vice versa. From this it follows that wherever appreciable flow exists, there is a true circulation, with an arterial supply and a venous return, so that neither the much quoted "venous reflux" nor the inflow of the blood into patent arteries (Moiroud [12]) plays an appreciable rôle after ligature. Such processes (venous reflux, flow of blood into patent arteries, collateral circulation) may at the very most increase the reddening and infarction, but they cannot cause these changes. On the basis of these experiments one is forced to reject the theory that collateral circulation (Sprengel,[3] Niederstein [4]) or venous reflux or flow of blood into patent arteries (Moiroud,[12] Litten,[1] Cohnheim [18]) is the cause of the infarction. On the other hand, the increased venous pressure and consequent changes in the vessels before the embolism occurs play a decisive rôle in the infarction, since the latter or, better, a similar condition can be produced experimentally by ligation of the portal vein or clinically by thrombosis of a mesenteric vein or by simultaneous venous and arterial thrombosis or by the strangulation of an intestinal loop—all processes in which the venous pressure is increased. Infarction after embolism is, so to speak, an allergic phenomenon.

It seems that collateral circulation is misused frequently in explanation of many pathologic processes—e. g., in explanation of pulmonary infarcts, intestinal infarcts and the necrosis of the liver which follows occlusion in the hepatic artery. In all these cases, when the occlusion occurs abruptly, the explanation should be sought rather in the abnormal vasomotor state of the vessels in the affected region.

18. Cohnheim, Julius: Gesammelte Abhandlungen, Berlin, A. Hirschwald, 1885, p. 381.

These experiments provide welcome support for my contention in an earlier paper [19] that the anatomic demonstration of the presence of collateral channels is not sufficient to warrant the assertion that collateral circulation is responsible for the presence or absence of any changes in the tissue unless such channels are present in sufficient number to make collateral circulation effective. In practice "sufficient" must be synonymous with "excessive."

SUMMARY

In experiments on rats, ligation of the superior mesenteric artery produced anemic necrosis; ligation of the portal vein caused a picture similar to hemorrhagic infarction. The fundamental difference between the results of the experiments on healthy animals and the result of clinical embolization of the superior mesenteric artery in a sick person is pointed out.

Microscopically, it is found in vivo that the cause of hemorrhagic infarction is neither venous reflux or arterial reflux nor collateral circulation. In man, anemic necrosis of the bowel does not occur after embolism, but an infarction analogous to a pulmonary infarct occurs because circulation with which one deals in embolism is not a normal but a failing one, which predisposes to stasis and hemorrhage. The cause of the hemorrhagic infarction is not to be sought in the neighborhood of the infarct but in the congested and altered state of the circulatory channels.

19. Loeffler, Louis: Arch. Path. **21**:496, 1936.

INHIBITORY ACTION OF SODIUM CHLORIDE ON SKIN NECROSIS PRODUCED BY STAPHYLOCOCCUS TOXIN

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A previous experiment [1] showed that rabbit's red blood cells suspended in a 5 per cent solution of sodium chloride are not lysed by staphylococcus toxin to the same degree as similar cells suspended in a physiologic solution of sodium chloride. It was suggested that this inhibition of lysis is the result of a change which occurs in the red cell in the presence of a hypertonic concentration of sodium chloride.

The intradermal injection of staphylococcus toxin produces skin necrosis in the rabbit. The skin might be appropriate to determine whether or not sodium chloride inhibits the necrotic action of staphylococcus toxin in a way comparable to the inhibition of lysis of rabbit's red cells. The present paper is a report of an experiment in which the skin necrosis produced by intradermal injection of staphylococcus toxin was inhibited by preliminary intradermal injection of a hypertonic solution of sodium chloride.

METHODS AND MATERIALS

Staphylococcus Used.—The strain of Staphylococcus aureus used for the production of toxin in this study was the same as that used in previous work.[2] This culture was obtained at autopsy from the pharynx of a patient with the clinical and pathologic lesions associated with agranulocytic angina.

Preparation of Toxin.—The toxin was obtained from a forty-eight hour growth of this organism in broth. The broth consisted of veal infusion, 2 per cent proteose peptone, 0.5 per cent sodium chloride, 0.7 per cent disodium hydrogen phosphate (Na_2HPO_4) and 0.3 per cent agar. This in 500 cc. amounts was put into flasks holding 2 liters each. The medium was then autoclaved. The inoculum for each flask consisted of 1 cc. of an eighteen to twenty-four hour infusion broth culture of the staphylococcus. Through a rubber stopper containing two small glass tubes, 20 per cent carbon dioxide in 80 per cent oxygen was slowly introduced into the flasks for from one to three minutes. The flasks were then sealed

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From the Department of Pathology, Vanderbilt University Medical School.

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1. Rigdon, R. H.: Inhibitory Influence of Hypertonic Solutions of Sodium Chloride on Staphylococcus Toxin, J. Infect. Dis., to be published.

2. Rigdon, R. H.; Joyner, A. L., and Ricketts, E. T.: Am. J. Path. **10**:425-433, 1934. Rigdon, R. H.: Arch. Path. **20**:201-208, 1935. Rigdon, R. H., and Leff, W. A.: ibid. **21**:298-307, 1936. Rigdon.[1]

by clamped rubber tubing and incubated at 37.5 C. for forty-eight hours. After centrifugation, the supernatant fluid was filtered through Berkefeld N or V candles, tested for sterility and stored at 15 C.

Experimental Technic.—The tests were made intradermally in twenty-six adult white rabbits. From three to five areas, measuring approximately 1.5 by 1.5 cm. on the right and on the left side of each animal, were subjected to intradermal injection of from 1.5 to 2.5 cc. of (right) an 0.85 and (left) a 5 per cent concentration of sodium chloride. Staphylococcus toxin in quantities of from 0.1 to 0.2 cc. was then injected into the blebs produced by the salt in turn at the following intervals of time: immediately, five, ten, fifteen, twenty, thirty minutes, one hour and two hours later. Necrotic areas were either measured or outlined on cellophane paper on several different occasions during the eight days in which the rabbits were under observation. When the necrotic areas were measured, the diameter was taken in two directions; when the areas were outlined on cellophane paper, the areas were measured in square centimeters with a planimeter.

LESIONS PRODUCED BY INTRADERMAL INJECTIONS OF STAPHYLOCOCCUS TOXIN

In normal rabbits the degree of local injury from staphylococcus toxin injected intradermally varies. Seven normal rabbits were given from 0.1 to 0.2 cc. of the toxin intradermally to determine the average extent of necrosis produced by this toxin. In these rabbits the dimensions of the inflammatory lesions varied. There was also a slight variation in the reactions in different portions of the same animal. After eighteen hours a diffuse inflammatory reaction and necrosis occurred at the point of injection. When the toxin was injected along the sides, the necrosis extended downward in the skin. When it was injected into the skin over the thighs, the necrosis extended posteriorly and inferiorly toward the rump. The acute inflammation began to subside after from forty-eight to seventy-two hours, and the necrotic areas were then outlined with greater accuracy. After from four to five days the surface of the necrotic areas was covered with a dry brown crust.

EFFECT OF PRELIMINARY INTRADERMAL INJECTION OF SODIUM CHLORIDE ON NECROTIC LESIONS

Necrosis occurred at the point of injection of the toxin in all the areas treated with sodium chloride in the concentration of 0.85 per cent except in one animal. Small areas of necrosis frequently occurred at the points of injection in areas which had been treated with sodium chloride in the concentration of 5 per cent. A second larger area of necrosis frequently occurred inferior to the site of injection in skin previously treated with the 5 per cent salt. These two areas of necrosis were separated by a zone of normal skin. The widest zones of normal skin were present in those animals in which the toxin was injected from ten minutes to one hour after the hypertonic salt solution. But when the toxin was injected immediately or two hours after the use of this

concentration of sodium chloride a single area of necrosis occurred at the site of injection. If the toxin was given five minutes after the salt, a single area of necrosis occurred in some rabbits and a double area in others.

The complete protocols on two rabbits will illustrate the typical skin lesions produced by the toxin following the intradermal injection of a hypertonic solution of sodium chloride:

Rabbit 1 was given an intradermal injection of 2 cc. of an 0.85 per cent solution of sodium chloride in five areas on the right side (fig. 1 *A*). On the left side an

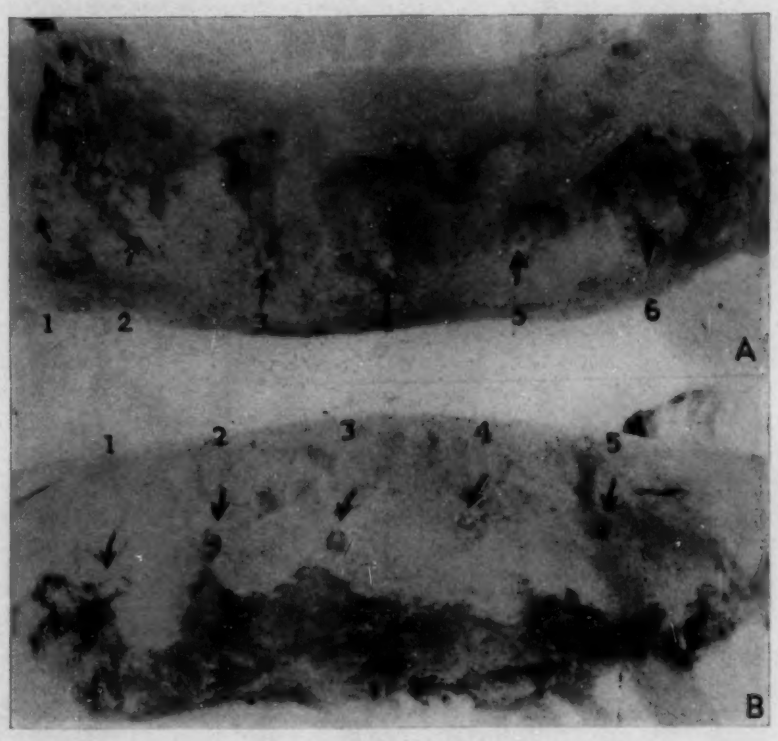

Fig. 1 (rabbit 1).—This rabbit was given 2 cc. of an 0.85 per cent solution of sodium chloride intradermally, on the right side, divided into four doses, each dose being injected over an area approximately 1.5 by 1.5 cm. *A* shows areas 1 to 5 on the right side. The same amount of a 5 per cent solution of this salt was injected similarly on the left side (*B*). The arrows indicate the points of injection of staphylococcus toxin at the following intervals: *1*, immediately; *2*, after five minutes; *3*, after ten minutes; *4*, after fifteen minutes; *5*, after twenty minutes. Arrow 6 in *A* indicates the point of injection of 0.1 cc. of toxin for control. The sides were photographed after twenty-four hours to show the hemorrhagic discoloration and beginning necrosis.

equal amount of a 5 per cent solution of sodium chloride was injected into areas that corresponded with those on the right side (fig. 1 *B*). Into each of the blebs

on both sides 0.1 cc. of toxin was injected in the following order: area 1, immediately; area 2, after five minutes; area 3, after ten minutes; area 4, after fifteen minutes, and area 5, after twenty minutes. Into area 6 on the right 0.1 cc. of toxin alone was injected, for the control.

Acute inflammatory and necrotic areas were observed in the skin on both sides of the rabbit after twenty-four hours. It was difficult to determine accurately the size of any necrotic area at this time. The location of the lesions relative to the points of toxin injection was distinctly different on the two sides. On the right, where the 0.85 per cent salt solution was injected, the areas of necrosis were continuous with the sites of injection of the toxin (fig. 1 *A*). On the left, where

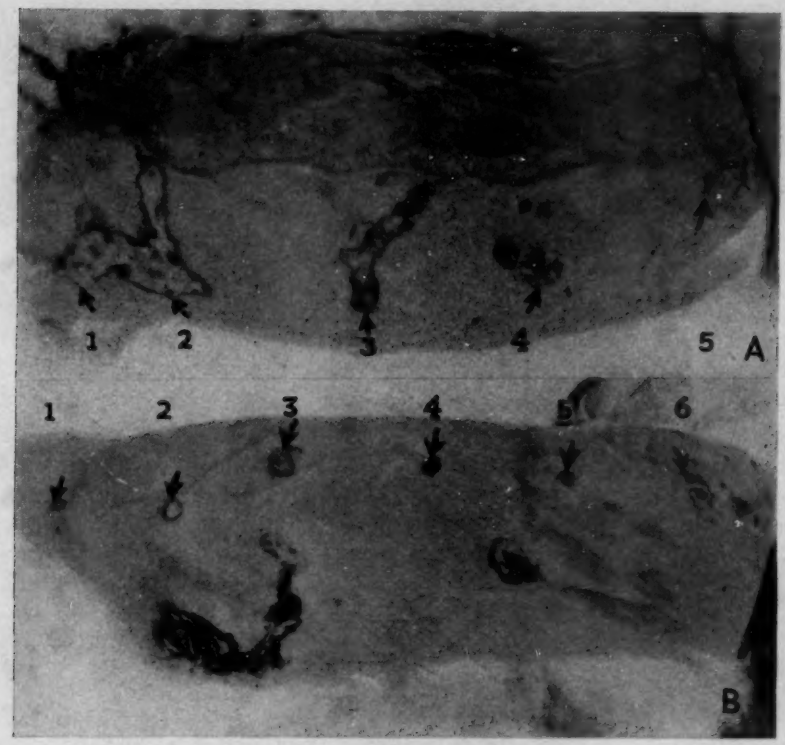

Fig. 2 (rabbit 2).—This rabbit received the same volume of each of the salt solutions and of the toxin in the same manner and at the same intervals as rabbit 1. Its sides (*A*, right; *B*, left) were photographed after seven days to show the location of the areas of necrosis with regard to the points of injection of toxin.

the 5 per cent solution was injected, necroses were present at the sites of toxin injection and also inferior to these points. The two regions of necrosis were separated by macroscopicallly normal skin (fig. 1 *B*). All the acute inflammatory lesions gradually disappeared, and thick brown crusts developed over the former areas of necrosis.

Rabbit 2 (fig. 2) was given injections of the same amounts of salt and toxin at the same intervals as rabbit 1. The early lesions in rabbit 2 were similar to those described for rabbit 1. Seven days after the injections of salt and toxin the

necrotic areas were outlined, and the necroses were recorded in square centimeters. The table shows that the necrosis which occurred at the sites of injection of toxin was less in the areas previously treated with 5 per cent sodium chloride than in the areas treated with 0.85 per cent salt solution. There was also necrosis in the skin on the left side inferior to that at the sites of injection of the toxin (fig. 2*B*). The skin between these two areas of necrosis was macroscopically normal.

ATYPICAL SKIN REACTIONS FOLLOWING INTRADERMAL INJECTIONS OF SODIUM CHLORIDE AND STAPHYLOCOCCUS TOXIN

The typical skin reactions following intradermal injections of sodium chloride solutions and staphylococcus toxin have been described for sixteen of the nineteen rabbits observed. Two rabbits with atypical skin reactions were obviously undernourished and dehydrated, and one had a diffuse red papilliferous skin lesion. In these two rabbits the areas

Areas of Necrosis in Rabbit 2 After Seven Days at Sites of Intradermal Injection of 2 cc. of Solution of Sodium Chloride Followed by Intradermal Injection of 0.1 cc. of Staphylococcus Toxin

Anatomic Location of Injections (Fig. 2)	Interval Between Injection of Salt and Toxin, Min.	Areas of Necrosis, Sq. Cm.	
		Right Side, 2 Cc. of 0.85 per Cent NaCl + 0.1 Cc. of Toxin	Left Side, 2 Cc. of 5 per Cent NaCl + 0.1 Cc. of Toxin
1 + 2	..	11.5	...
1	Immediate	....	0.5
2	5	....	0.5
3	10	4.0	1.0
4	15	2.5	0.5
5	20	0.25	0.25
6	Control	....	1.5

of necrosis were all larger than those in the animals previously described, although the two rabbits were given the same amounts of salt and toxin. Confluent necrotic lesions occurred on the left side, where the 5 per cent salt solutions and toxin were injected. In the rabbit with the dermatitis the necrosis on the right and left sides extended upward over the midline of the back. One rabbit in the group of nineteen showed no necrosis. Furthermore, no necrosis occurred in this animal in the area receiving the injection of 0.1 cc. of toxin alone. This probably was an immune animal.

COMMENT

The nature and extent of the skin lesion occurring in the rabbit following the intradermal injection of staphylococcus toxin alone have been described by others.[3] The variations observed may represent immune reactions and apparently are not related to the inhibition which

3. Parker, Julia T.: J. Exper. Med. **40**:761-772, 1924. Burnet, F. M.: J. Path. & Bact. **32**:717-734, 1929.

is produced by the preliminary injection of a hypertonic solution of sodium chloride. Salt in the concentration used in this study did not produce necrosis and rarely any local hyperemia within twenty-four hours.

The experiments reported here show that a preliminary intradermal injection of 2 cc. of a 5 per cent solution of sodium chloride protects the skin against the necrosis produced by an intradermal injection of 0.1 cc. of staphylococcus toxin. The maximum degree of protection from sodium chloride in this concentration and volume is obtained when the interval between the injections of salt and toxin is not less than five minutes or more than one hour.

Rabbits differ in their response to the intradermal injection of staphylococcus toxin. It is therefore necessary to use a large number of animals to establish the maximum and the minimum period of time in which to obtain the greatest degree of protection with a definite concentration and volume of the salt and of the toxin. Other factors may also influence necrosis, namely, the sodium chloride content of the blood, the nutrition, and the fluid balance of the body. This was suggested by the two rabbits which gave the atypical skin reactions.

Complete inhibition of the necrosis at the site of injection of the toxin is rarely produced by the intradermal injection of 2 cc. of a 5 per cent solution of sodium chloride. This amount of the salt solution usually, however, produces a decrease in the extent of the necrosis at the point of injection as shown in the table. In the areas treated with 2 cc. of a 5 per cent solution of salt there are frequently areas of necrosis inferior to those which occur at the sites of injection. These two regions of necrosis are separated by normal skin. The amount of normal skin separating the two regions is apparently influenced by the interval of time between the injection of the salt and that of the toxin.

There is some evidence to show that a preliminary injection of 2 cc. of an 0.85 per cent solution of sodium chloride inhibits the skin necrosis produced by staphylococcus toxin. The degree of inhibition of necrosis, however, is small. One rabbit in this series treated with salt in this concentration and one hour later with the toxin showed a decrease in the amount of necrosis at the site of injection and an area of necrosis inferior to this point. This lesion resembled those produced in rabbits following the injection of 5 per cent salt solution. The exact effect of 2 cc. of an 0.85 per cent salt solution on the necrosis produced by 0.1 cc. of the toxin cannot be determined from the present data. It appears, however, that the amount of necrosis decreases with an increase in the concentration of sodium chloride up to 5 per cent. Salt above this concentration produces some injury to tissue cells.

The inhibitory action of sodium chloride on the dermal necrosis produced by staphylococcus toxin may be the result of the action of

the salt on the tissue cells. The change effected may inhibit the union of the toxin and the cells. This union apparently is responsible for the necrosis. Staphylococcus toxin is apparently not neutralized by sodium chloride in the tissue, for necrosis frequently occurs inferior to the point of injection of the toxin in areas previously treated with a hypertonic concentration of salt. In this connection it is interesting to know that sodium chloride added directly to staphylococcus toxin in a quantity sufficient to make a 12 per cent concentration does not destroy the hemolytic action.[1] Dilution or neutralization of the toxin by fluids apparently affords no explanation for this inhibitory phenomenon. The maximal edema is present immediately after the injection of the salt solution. The injection of toxin at this time produces a single large area of necrosis at the point of injection.

SUMMARY

Nineteen rabbits were treated intradermally in five areas on the right and on the left side with from 1.5 to 2.5 cc. of an 0.85 per cent (right) and a 5 per cent (left) solution of sodium chloride. Into each bleb made by the salt solution, from 0.1 to 0.2 cc. of staphylococcus toxin was injected, in turn, at intervals varying from immediately to two hours. Sixteen rabbits showed a decrease in the extent of necrosis in the areas previously receiving the 5 per cent salt solution. The most pronounced degree of inhibition occurred when the interval between the injection of the 5 per cent salt solution and the injection of the toxin was from five minutes to one hour. The location of the necroses with regard to the points of toxin injection in the areas receiving the one concentration of sodium chloride was different from that in the areas receiving the other. In the areas treated with the 5 per cent salt solution necrosis occurred at the sites of toxin injection and also, frequently, inferior to these points. The skin between these two necrotic regions was macroscopically normal. The inhibitory action of sodium chloride on the dermal necrosis produced by staphylococcus toxin may be the result of the action of the salt on the tissue cells. The change effected may inhibit the union of the toxin and the cells.

EXPERIMENTAL PULMONARY ABSCESS IN MONKEYS

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AND
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A review of the recent literature on the etiology and pathogenesis of chronic pulmonary abscess [1] reveals a diversity of opinion and a need for further investigation. Several writers [2] have concluded that the disease may result from aspiration into the bronchi of infectious material derived from the oral cavity or the upper respiratory passages. Others,[3] who were unable to reproduce the infection experimentally in this manner, have expressed the point of view that embolism is the usual cause. As pointed out by Lauche [4] and by Marshall and Brunn,[5] it is quite possible that in some cases the one, while in other cases the other, mode of infection is involved.

Because of the low incidence of this disease in general hospital practice,[6] it has been difficult, if not impossible, to obtain material and to study the disease in its early stages. It seemed to us essential therefore, first, to develop a method of reproducing the infection at will in the laboratory and, second, to select an experimental animal with an anatomy which closely approximates that of man and, particularly, with a bronchial tree which is vertical rather than horizontal. It is for this reason that we shall report, for the first time, to our knowledge, the production of pulmonary abscess in monkeys.

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1. (*a*) Maxwell, J.: Quart. J. Med. **3**:467, 1934. (*b*) Graham, E. A.: Singer, J. J., and Ballon, H. C.: Surgical Diseases of the Chest, Philadelphia, Lea & Febiger, 1935, p. 709.

2. (*a*) Smith, D. T.: Oral Spirochetes and Related Organisms in Fuso-Spirochetal Disease, Baltimore, Williams & Wilkins Company, 1932. (*b*) Scarff, J. E.: Arch. Surg. **18**:1960, 1929.

3. Cutler, E. C.: Am. J. Dis. Child. **38**:683, 1929. Longacre, J. J., and Herrmann, L. G.: Arch. Surg. **30**:476, 1935.

4. Lauche, A.: Die Entzündungen der Lunge und des Brustfelles, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1928, vol. 3, pt. 1, pp. 845-864.

5. Marshall, M. S., and Brunn, H.: Arch. Surg. **26**:225, 1933.

6. Brunn, H.: J. A. M. A. **103**:1999, 1934.

METHODS AND MATERIALS

Experimental Animals.—Java monkeys (Macacus irus) were kept in individual clean cages on a suitable mixed diet and carefully observed for several weeks prior to inoculation.

Materials Used for Inoculation.—Bits of pulmonary tissue removed by Dr. Harold Brunn from a patient with chronic pulmonary abscess at the Mount Zion Hospital were injected subcutaneously into the inguinal tissues of several guinea-pigs.[7] The various micro-organisms, which were present in the resulting suppurative lesion, including Bacterium melaninogenicum, several types of fusiform organisms and spirochetes, anaerobic nonhemolytic streptococci and gram-negative motile and nonmotile rods of the Bacteroides group were kept alive by frequent passage through guinea-pigs. After ten such transfers the aerobic micro-organisms had disappeared.

Technic of Inoculation.—Each of a number of monkeys which had fasted for eighteen hours was given ½ grain (0.032 Gm.) of morphine sulfate subcutaneously, placed on a special animal board [8] and carried to the fluoroscopic room. After the animal had been anesthetized with ether, a small sand-bag was placed under the lumbar spine to extend the head. The mouth was cleansed with gauze, and the throat and larynx were painted with a 10 per cent solution of procaine hydrochloride in order to abolish temporarily the cough reflex. By means of a mouth gag, a tongue forceps and a retractor which pushed the tongue downward and forward, the larynx was exposed and under direct vision a no. 5 radiopaque ureteral catheter was introduced into the bronchus of the lower lobe of one lung, preferably the right. The position of the catheter was checked with the aid of the fluoroscope, and infectious material was then injected directly through it by means of a needle and a Luer syringe. After the position of the catheter in the bronchus was rechecked, the animal was held in an upright position for several minutes and then returned to its cage.

This technic is a modification of that described by Terrell, Robertson and Coggeshall [9] and by Francis and Terrell [10] in their work on experimental lobar pneumonia in dogs.

Obstruction of the Bronchus.—After repeated failure to infect any monkey when the bronchus was not intentionally blocked, a technic was developed which prevented the expulsoin of the injected material. This consists in the intrabronchial injection through the radiopaque catheter of from 1.5 to 2 cc. of a 2.5 per cent solution of melted agar (kept warm at 40 C.) immediately after the introduction of the infectious material.

Roentgenographic Technic.[11]—Roentgenograms, including lateral and anteroposterior views, were taken of all animals before the injection of pus and at frequent intervals thereafter. A portable Coolidge machine and a Victor tube were employed. The target field distance was 30 inches (86 cm.); the amperage, 30 milliamperes; the exposure, a tenth of a second.

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7. Kline, B. S.: J. Infect. Dis. **32**:481, 1923. Smith.[2a]

8. Wu, C.: Radiology **19**:215, 1932.

9. Terrell, E.; Robertson, O., and Coggeshall, L.: J. Clin. Investigation **12**: 393, 1933.

10. Francis, T., Jr., and Terrell, E.: J. Exper. Med. **59**:609, 1934.

11. Dr. Joseph Levitin, associate chief of roentgenology of the Mount Zion Hospital, collaborated in this phase of the work.

Clinical Records and Necropsies.—Rectal temperatures and blood counts were recorded at frequent intervals before and after the injections, and the animals were observed for appetite, cough and general behavior. Examinations were made as soon after death as possible. Cultures were taken of the cardiac blood and of various portions of the lungs and other viscera.

LESIONS PRODUCED

Fourteen monkeys were given intrabronchial injections in the manner described. Of these, six died at intervals of from two to seventeen days after infection, while others were killed for observation at intervals of from eight to seventy-four days. Large, putrid abscess cavities, occupying almost wholly the involved lobes of the infected lungs were found in six of the animals, which had been inoculated from two to forty-six days previously. One monkey died of pneumonia on the fourth day; another sick animal which was killed was found to have a similar condition. In two other animals which were killed twenty-three and thirty-seven days, respectively, after injection of the pus, the lungs showed small firm areas infiltrated with agar and surrounded by zones of inflammation. No significant pulmonary lesions were observed in four other monkeys at necropsy.

A series of fifteen animals, divided into four groups, served as controls. Nine of these were given intrabronchial injections of pus which had been previously sterilized by heating at 100 C. for one-half hour, while two received sterile broth. In all of these monkeys, the bronchi were plugged by means of melted agar. Four others received only the agar plug.

None of the animals of the control series showed any signs of illness after this treatment. Two of the last group showed transitory processes at the base of the right lung in the roentgen films. Animals which were killed during the first forty-eight hours after the injections showed atelectasis and occasionally slight bronchiectasis. There was also increased firmness of the lung tissue due to infiltration with agar and the inflammatory reaction which resulted therefrom. The agar could not be found in the bronchial tubes, since it had infiltrated into the alveoli, as shown by histologic studies. There was no evidence of pulmonary suppuration or of cavity formation in any of these control animals. The details of the pathologic observations are given in a subsequent section of this paper.

The following protocols are cited as illustrative of the lesions obtained:

Experiment 1.—M. irus 19 was given an injection April 15, 1935: 0.2 cc. of pus followed by 2 cc. of a 2.5 per cent solution of agar to the lower lobe of the left lung. The animal was coughing at this time, since it had previously received three intrabronchial injections of powdered emery in an attempt to lower its

resistance to infection. Four days later the roentgen film showed increased density at the base of the left lung posteriorly. The animal died May 1, sixteen days after the injection of pus, at which time the lesion had spread into the upper lobe of the right lung. At autopsy there were scattered fibrous and fibrinous adhesions in the left pleural cavity, mainly at the base of the lung, with a few pleural adhesions on the right side. The left lower lobe was replaced by a large putrid abscess cavity of dumb-bell shape, 2 cm. in its greatest dimension. The cavity was surrounded by a zone of pneumonitis and communicated directly with a bronchus. The walls were rough, grayish and necrotic and were about 2 or 3 mm. thick. The right upper lobe showed superficial atelectasis. With the dark field numerous spirochetes, various fusiform organisms, rods and cocci were seen. Fusiform organisms, Bact. melaninogenicum, anaerobic streptococci and unidentified micrococci as well as several aerobic gram-negative diplococci and Streptococcus viridans were isolated from these lesions.

Experiment 2.—M. irus 79 was given an injection Nov. 13, 1935: 1 cc. of pus followed by 1.5 cc. of a 2.5 per cent solution of agar to the left lung. Two days after injection a slight febrile reaction was noted together with a sharp drop in the total leukocyte count, which seemed to be at the expense of the lymphocytes. The number of nonfilamented forms increased markedly during the first two days after injection but returned subsequently to normal (fig. 1). On the fifth day after injection of the pus the animal coughed a good deal and its breath was very foul. It died suddenly. One-half hour prior to death, the x-ray film demonstrated an area of increased density which had been growing progressively since the time of inoculation. At autopsy, there was a large quantity of foul sputum in the mouth. The lower lobe of the left lung was replaced by a large cavity containing a foul-smelling brown liquid. The pleura was adherent to the diaphragm, and there was pneumonitis surrounding the cavity. The right lung seemed normal. The spleen was enlarged and congested. The trachea contained putrid material. The bacteriologic studies showed the usual flora of abscess of the lung, including spirochetes, fusiform organisms, etc.

Experiment 3.—M. irus 5 had received insufflations of powdered cement on March 19, 21 and 28 and April 11, 1935, and a mild cough had developed. On April 15, the lower lobe of the left lung received an injection of 1 cc. of fresh pus from a guinea-pig, followed by 1 cc. of melted agar. From April 19 until May 1 x-ray films revealed pneumonia at the base of the left lung posteriorly. On May 9 and May 17, the roentgenologist diagnosed an abscess at the base of the left lung, surrounded by pneumonic infiltration. The animal ate very poorly and coughed frequently. It showed no marked febrile reaction, however. On May 31, it was put to death for study. The left lower lobe was found adherent to the diaphragm and to the pleural wall posteriorly. The lung was consolidated and grayish black; when it was cut, pus oozed out. The agar block was found in the dilated bronchus, and there was atelectasis of the corresponding lobe.

SUMMARY OF BACTERIOLOGIC OBSERVATIONS [12]

Direct examination of the bits of lung tissue removed at operation from the patient with chronic pulmonary abscess showed either with the dark field of the microscope or in stained preparations the following

12. Mrs. D. G. Mercado and Mrs. R. M. Barnhart collaborated in the bacteriologic studies.

micro-organisms: various types of motile spirochetes, gram-negative vibrio-like organisms, gram-negative cocci and threadlike organisms, gram-positive cocci in pairs or chains, short and long gram-positive rods and tiny gram-negative coccobacilli. In aerobic cultures Streptococcus haemolyticus-beta, Staphylococcus aureus (hemolytic) and Staphylococcus albus were recovered, while the anaerobic flora included Bact. melaninogenicum, Bacillus fusiformis, indifferent streptococci and a very small gram-negative motile rod.[13]

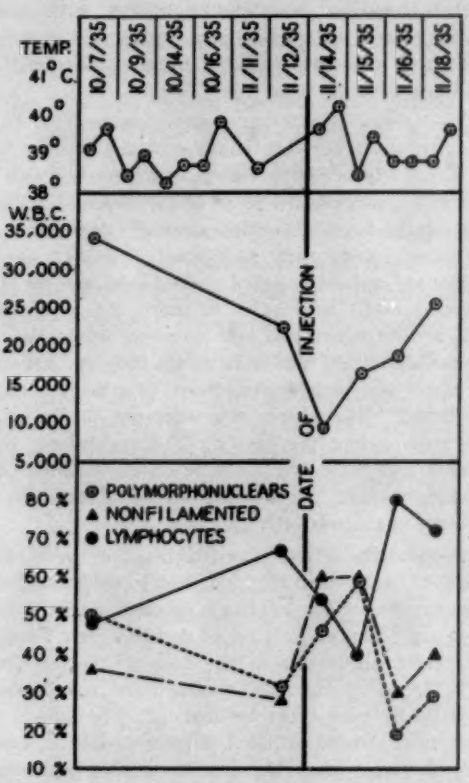

Fig. 1 (M. irus 79).—Changes in temperature and in leukocyte counts after intrabronchial injection of a purulent exudate containing anaerobic bacteria, followed by a plug of agar.

As illustrative of the micro-organisms found in experimental lesions in monkeys, the following protocols are cited:

M. Irus 19.—Dark field examination of pus removed from the left lung at autopsy showed spirochetes, fusiform-like organisms, cocci and actively motile rods. The cardiac blood contained anaerobic streptococci. Cultures from the

13. Footnote deleted by the authors.

abscess cavity yielded Strep. viridans, gram-negative diplococci, fusiform organisms, chains of anaerobic streptococci, Bact. melaninogenicum, a gram-negative micrococcus and other anaerobic gram-positive cocci.

By removing blood from the saphenous vein at various intervals after intrabronchial injection of pus [14] it was observed that in two of the animals (80 and 81) several types of bacteria, including anaerobic streptococci, fusiform organisms and anaerobic gram-negative motile and nonmotile rods of the Bacteroides group had passed the barrier of the pulmonary tissues and penetrated into the circulation as early as ten or fifteen minutes after infection. Bact. melaninogenicum and spirochetes were absent from the peripheral blood at this time, but all, spirochetes excepted, appeared twenty-four hours later. There was also an occasional transitory bacteremia in animals in which pulmonary lesions failed to develop. However, only blood from monkeys which suffered severe pulmonary injury gave positive cultures persistently.

At autopsy, the cardiac blood of monkeys with pulmonary abscesses often showed an encapsulated anaerobic streptococcus. The abscess cavity had the usual flora. From the liver and spleen, fusiform organisms, gram-negative motile and nonmotile anaerobic bacilli and anaerobic streptococci were cultivated. In the lungs of animals in which suppurative lesions of the lung failed to develop, spirochetes, fusiform organisms, various types of Neisseria and staphylococci were found, but careful investigation revealed that these represented the normal flora of their upper respiratory passages.

HEMATOLOGIC, ROENTGENOLOGIC AND CLINICAL DATA

It was not possible to observe any constant changes in the temperature or blood picture of animals after infection. Monkeys in which abscesses of the lung developed usually showed a moderate but transient increase in temperature, up to 40.2 C. (104.3 F.). The white blood cell counts occasionally fell quickly to as low as 2,000 per cubic millimeter in animals in which the process was rapidly fatal, but returned to normal levels in monkeys which survived a little longer (fig. 1). The number of nonfilamented polymorphonuclears increased slightly. In animals which failed to show pulmonary lesions there was likewise no significant change in temperature. The leukocytes, particularly the polymorphonuclears, increased in number slowly, the nonfilamented forms being most prominent. There was, however, a gradual return to normal.

Control animals (48, 55 and 56) given an injection of agar only had a slight increase in temperature and a transitory mild decrease in the lymphocyte and total leukocyte counts. The number of nonfilamented polymorphonuclears remained high for several days after injection. In four other controls (82, 83, 84, 94) receiving heated pus followed by agar the temperature changes were insignificant. Three of them revealed a transient drop in the polymorphonuclear and total white cell counts with a relative increase in the number of nonfilamented forms.

14. Weiss, C.: J. Immunol. **30**:394, 1936.

The roentgen findings were similarly inconstant. For only two animals (5 and 19) was it possible to make a diagnosis of pulmonary abscess during life (fig. 2). Reports on roentgenograms usually read, "increased density, probable pneumonia or atelectasis," in spite of the fact that the autopsies revealed relatively large abscess cavities. This is in harmony with experience in clinical medicine.[15]

Since there is great need for a laboratory method of diagnosing this disease in its early stages, we are conducting investigations on the

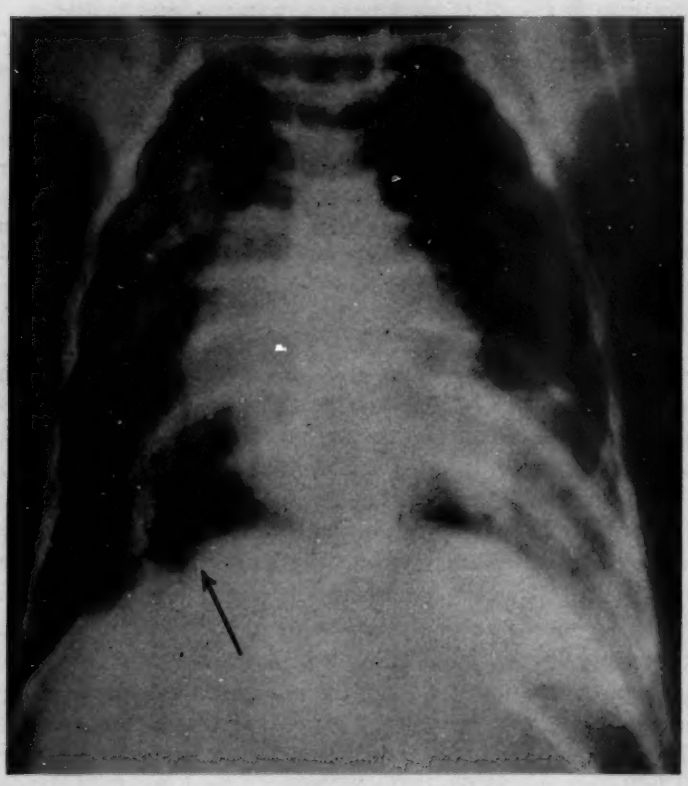

Fig. 2 (M. irus 19).—Roentgenogram (anteroposterior view) showing an abscess in the lower lobe of the right lung (April 29, 1935).

development of serum precipitins for the nucleoproteins and specific carbohydrates derived from various aerobic and anaerobic bacteria associated with chronic suppurations of the lung.[16]

15. Lerio, N.: Ergebn. d. inn. Med. u. Kinderh. **42**:376, 1932.

16. Weiss, C., and Czarnetzky, E. J.: Proteins and Carbohydrates Derived from Anaerobic Bacteria, to be published.

SUMMARY OF PATHOLOGIC FINDINGS [17]

As indicated in the foregoing protocols, it was found necessary to obstruct the bronchus receiving the injection of pus in order to prevent the expulsion of the inoculum during cough. The agar which was employed for this purpose acted as a foreign body irritant and produced an acute inflammatory reaction characterized by cellular proliferation, edema and formation of foreign body giant cells. In no instance, however, was there any sign of tissue necrosis or of pulmonary suppuration. Phagocytosis by monocytes and giant cells served to dispose of this foreign material; healing resulted, and the animals remained perfectly well. In marked contrast to these controls were the monkeys which received fresh, unheated pus containing a variety of anaerobes. Six of a series of fourteen presented abscesses while two had pneumonia. A few illustrative protocols may now be cited.

M. Irus 19.—This animal died sixteen days after infection, and in the lower lobe of the left lung a large necrotic abscess cavity was found which communicated with the bronchus. Histologic sections showed a well organized fibrous wall with evidence of an inflammatory response by cells, including giant cells. There were also several circumscribed areas of necrosis and diffuse polymorphonuclear infiltration.

The diagnosis was pulmonary abscess with chronic interstitial pneumonia and fibrosis.

M. Irus 81.—This monkey died two days after injection, showing putrid abscesses of the lower lobe of the right lung with pneumonitis of the remaining portion of the lung. Microscopic examination revealed most of the sections to be completely filled with a seropurulent exudate. The alveolar walls could still be distinguished. In some regions an opaque nonstaining substance (agar) was present in the alveoli. There were fat-containing phagocytic cells in the exudate which filled most of the large and small bronchioles. Some sections showed marked atelectasis without inflammation, while a fibrinopurulent pleuritis extended over the pneumonic region.

The diagnosis was pulmonary abscess and acute diffuse pneumonitis with accompanying fibrinopurulent pleuritis.

M. Irus 128 (Control).—This animal was given an intrabronchial injection of agar only and killed forty-eight hours later. At autopsy the right lung showed a normal smooth surface, with some old fibrous adhesions extending to the diaphragm and the mediastinal pleura. The lower lobe was increased in size and density and was grayish. On sectioning, it was beefy in appearance and atelectatic. The agar plug was not found on dissection of the bronchi.

Microscopic examination of this lobe showed most of the alveoli markedly distended and completely filled with a eosinophilic homogeneous mass (agar). In many instances desquamated epithelial cells and polymorphonuclear leukocytes were noted within the homogeneous masses, here and there mingled with red blood cells. Most of these cellular elements were at the periphery of the alveoli. Occasionally there was a foreign body giant cell.

17. Dr. G. Y. Rusk, Dr. G. R. Biskind and Dr. K. Neugarten of the department of pathology of the Mount Zion Hospital collaborated in this phase of the work.

Some of the alveolar septums had ruptured, while others showed marked widening due to congestion of the interalveolar capillaries and edema which separated the delicate connective fibers of the septums. Within the capillaries and migrating into the alveolar walls a great number of polymorphonuclear leukocytes could be seen. They continued into the alveoli, forming large accumulations. At the periphery of the lung there was a zone consisting of alveoli filled with an edematous fluid and cellular material (polymorphonuclears). The alveolar walls could be clearly made out. The capillaries accompanying the septums were slightly less congested; their endothelial linings, however, were more swollen. The alveolar epithelial lining was also markedly swollen or desquamated.

Further toward the periphery huge vacuolated spaces, due to rupture of the alveolar septums, were encountered. Other small alveoli were filled with fluid in which a moderate number of white blood cells and red blood cells were seen. There was no evidence of desquamation of alveolar epithelium at this site. The epithelium of the bronchi was usually intact, but in some regions single epithelial cells or clusters of from 3 to 5 had broken loose from the wall and were lying within the lumens. Some of the latter were almost completely filled with agar, while others contained leukocytes and erythrocytes.

Sections from the upper lobe of the right lung as well as those from the left were normal. Sections from the heart, kidneys, liver and spleen likewise showed no pathologic changes. Within the spleen there were, however, a few follicles which contained large germinal centers.

M. Irus 129 (Control).—The animal was given an intrabronchial injection of agar only and killed seven days later. At autopsy the pleurae and pleural cavities were normal. The lower lobe of the right lung did not retract when the pleural cavity was opened. On section, the lobe was firm in consistency, grayish red, with granularity and dryness of the cut surface, and atelectatic. There was slight dilatation of the bronchus leading to this lobe. The agar had infiltrated the lung tissue and could not be found in the bronchus. There was no edema of the bronchi.

Microscopic examination of the section of the involved lobe showed most of the alveoli filled with an eosinophilic homogeneous substance (agar). Here and there within the smaller or larger alveolar spaces lymphocytes and epithelial cells were noticed. There was also a remarkable number of foreign body giant cells present, which lay mostly at the border of the homogeneous masses. These cells protruded into the intra-alveolar substance and seemed to resorb this material. The giant cells had 8 or 10 and occasionally more than 20 nuclei. Sometimes they included phagocytosed leukocytes and other nuclear débris. In some regions a few degenerating leukocytes were seen.

The interalveolar septums contained between their delicate connective tissue fibers a few leukocytes and lymphocytes. In addition, regeneration of the alveolar epithelium was visible. The blood vessels of the alveolar septums were compressed and only in few instances patent. The bronchi showed well preserved epithelium and sometimes contained a few white and red blood cells. The larger bronchi were often surrounded by small foci of lymphocytes. Regions farther toward the periphery showed the alveoli almost completely filled with desquamated swollen epithelial cells intermingled with polymorphonuclear leukocytes and occasional giant cells.

The zone directly beneath the pleura showed emphysematous alveoli alternating with atelectatic ones. In the latter mainly, a slight inflammatory exudate could

be noticed which was less prominent than that found in the adjacent zone. Here also the intra-alveolar elements consisted mainly of desquamated epithelial cells, between which a few leukocytes and monocytes could be seen.

Sections from other parts of the lungs and from the heart, kidney, liver and spleen were normal.

COMMENT

Pulmonary abscess differs from other inflammation of the lung in that lung tissue is destroyed and liquefied or disappears entirely with the production of a cavity. When the content of the latter is putrid and there is no line of demarcation between the diseased and the relatively normal tissue, one speaks of pulmonary gangrene.[4] Clinically, this is a fulminating process, associated with toxemia, and usually involves an entire lobe of the lung. The exact cause of this destructive process is not yet known. It may be referable to the toxic products or proteolytic enzymes of various aerobic or anaerobic micro-organisms, to a disturbance of the balance between the proteolytic enzymes and the anti-enzymes of the leukocytes and the serum of the inflammatory exudate [18] or to other, as yet unknown, causes. Factors such as thrombosis of blood vessels, circulatory stasis and decreased amplitude of respiration occasioned by insufficient movements of the diaphragm after operation and trauma [19] have been mentioned by several writers.[20]

Because of the need of studying the disease in its early stages and under experimental conditions, we have developed a method of reproducing abscess of the lung in monkeys by the intrabronchial injection of a mixture of anaerobic organisms, including Bact. melaninogenicum, various fusiform organisms, anaerobic streptococci, vibrios and various gram-negative bacilli of the Bacteroides group, the virulence of which has been maintained or increased by repeated passage through animals. It must be emphasized that successful results depend on obstruction of the infected bronchus—a procedure which traps the infectious material in the smaller divisions of the bronchial tree and probably also in the alveoli. Additional effects probably are: (*a*) the production of atelectasis [21] and decreased arterial circulation in that portion of the lung [22] and (*b*) interference with the normal peristaltic contractions of

18. Weiss, C., and Czarnetzky, E. J.: Arch. Path. **20**:233, 1935.

19. Razemon, P.: Compt. rend. Soc. de biol. **100**:878, 1929.

20. Van Allen, C. M.; Adams, W. E., and Hrdina, L. S.: Arch. Surg. **19**: 1279, 1929. Tuttle, W. M., and Nicoll, G. L.: J. Thoracic Surg. **2**:60, 1932.

21. Garcia-Otero, J.; Barcia, P. A., and Volonterio, M.: J. de radiol. et d'électrol. **19**:441, 1935. Lee, W. E.; Tucker, G., and Clerf, L.: Ann. Surg. **88**: 6, 1928. Coryllos, P. N., and Birnbaum, G. L.: Arch. Int. Med. **51**:290, 1933.

22. Coryllos, P. N., and Birnbaum, G. L.: Arch. Surg. **19**:1346, 1929. Andrus, W. C.: ibid. **10**:506, 1925; quoted by Graham, Singer and Ballon.[1b]

the bronchi and with the activity of the bronchial cilia, which aid in the expulsion of foreign material, including bacteria.[23]

Histologic sections showed that the material (agar) employed for obstructing the bronchus acts as a foreign body irritant, producing inflammation, cellular proliferation, exudation and finally fibrosis and healing, but never tissue necrosis. Of fifteen control animals which were given injections of agar, none produced pulmonary abscess; while, of fourteen which were infected with a mixture of anaerobes contained in a purulent exudate, followed by an agar plug, six showed pulmonary abscess and two pneumonia. At autopsy, in addition to the large abscess cavities, there was pneumonitis with an interstitial inflammatory reaction extending into the septums. This might therefore be characterized as an acute purulent necrotizing process. When the monkeys survived a sufficiently long time, a fairly definite fibrous wall was formed around the abscess cavity. This was especially noticeable in dogs, which were used in another series of experiments.[24] Whether this phenomenon is referable to local tissue immunity [25] or to allergic or hyperergic phenomena [26] remains to be determined.

Investigations now in progress in this laboratory suggest that previous damage of the lungs and other tissues by a bacterial toxin such as that of Staph. aureus may enable opportunists or "half-parasites," [27] including Bacillus fusiformis, Bact. melaninogenicum, anaerobic streptococci and various gram-negative bacilli of the Bacteroides group, which inhabit the upper respiratory tract, to become invasive and destroy portions of a lung. Bact. melaninogenicum possibly accomplishes this by means of its potent fibrinolysin.[28]

SUMMARY

A method is described for the production of pulmonary abscess in Java monkeys (M. irus) by the bronchial route. The infectious material was obtained at operation from the lung of a patient with chronic pul-

23. Linton, C. S.: Ann. Otol., Rhin. & Laryng. **42**:64, 1933. Moise, T. S., and Smith, A. H.: Arch. Int. Med. **45**:92, 1930.

24. Weiss, C.; Goldman, A., and Shevky, M. C.: Arch. Surg. **33**:649, 1936.

25. Fried, B. M.: Arch. Path. **17**:76, 1934. Stuppy, G. W.; Cannon, P. R., and Falk, I. S.: J. Prev. Med. **5**:97, 1931. Walsh, T. E., and Cannon, P. R.: Arch. Otolaryng. **20**:820, 1934. Tuttle, W. M., and Cannon, P. R.: Arch. Path. **16**:306, 1933. Sullivan, F. L.; Neckermann, E. F., and Cannon, P. R.: J. Immunol. **26**:49, 1934.

26. Kurotchkin, T. J., and Lim, C. E.: Proc. Soc. Exper. Biol. & Med. **31**: 1332, 1933. Rössle, R.: Verhandl. d. deutsch. path. Gesellsch. **19**:18, 1923. Gerlach, W.: Virchows Arch. f. path. Anat. **247**:295, 1923.

27. Gundel, M.; György, P., and Pagel, W.: Ztschr. f. Hyg. u. Infektionskr. **113**:629, 1932.

28. Weiss, C.: Unpublished data.

monary abscess, and the anaerobes which it contained (Bact. melaninogenicum, spirochetes, various types of fusiform organisms and gram-negative bacilli of the Bacteroides group, as well as anaerobic streptococci, etc.) were kept alive by frequent passage through the inguinal tissues of guinea-pigs. With the aid of a fluoroscope and a radiopaque ureteral catheter, the purulent infected exudate which was removed from the inguinal lesions of the guinea-pigs was introduced directly into the bronchus of the lower lobe. The bronchus of one lung was then plugged by means of melted agar in order to prevent expulsion of the bacteria during cough.

In six of fourteen animals thus infected there developed large putrid abscesses and in two pneumonia, while in the rest the results were essentially negative. The progress of these lesions was followed during life by frequent roentgen examinations and checked by means of histologic sections at necropsy.

Control experiments with heated pus and agar or agar alone produced atelectasis and pneumonitis but no evidence of pulmonary suppuration.

In only two of the six monkeys which showed abscesses at autopsy was it possible to diagnose the condition by means of x-ray films during life. This is similar to the usual clinical experience.

The hematologic and temperature changes likewise were not sufficiently constant to be of diagnostic value.

Bacteriologic and histologic examinations were made of the organs of monkeys with experimental pulmonary abscesses and of control animals. The details are given in the text.

The evidence presented suggests that pulmonary abscess may be due in certain cases to aspiration into the bronchioles or alveoli of infectious material derived from the upper respiratory passages and plugging of the corresponding bronchus. The rôle of pulmonary atelectasis and of bacterial toxins in paving the way for saprophytes and opportunists is discussed.

RAPID, RELIABLE AND SIMPLE TECHNIC FOR SIMULTANEOUS DEHYDRATION AND INFILTRATION OF HUMAN TISSUES

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The satisfactory embedding of an animal object or human tissue in paraffin requires the removal of water by passage through a large series of alcohols of increasing concentration and clearing by intermediate fluids, such as chloroform and xylene, freely miscible with alcohol. Embedding in pyroxylin (celloidin), although slow in point of time, is invaluable for certain soft or brittle tissues, but the cutting of pyroxylin sections is laborious. Embedding in pyroxylin and paraffin as usually practiced is also tedious, but cutting is relatively easier. These technics demand the constant attention of technicians and are time-consuming. Since the introduction of paraffin as an embedding medium by Klebs [1] in 1869 many labor-saving devices and technics simplifying tissue dehydration and infiltration have been proposed. Because those now in use are admittedly imperfect, he who offers a new technic need not do so apologetically.

I submit a histologic technic which not only dehydrates and infiltrates tissues simultaneously but also possesses certain other substantial improvements on the technical procedures now available. It not only carries out the primary purpose of hardening by conferring on tissues sufficient consistence to permit further manipulation without distortion, but also, by its withdrawal of water so necessary to the active process of life, arrests bacterial growth and permanently preserves the preparation. It satisfies the following requisites of a superior histologic technic: (1) reliability, facility and simplicity; (2) reduction of time in operation; (3) increased rapidity and superior definition of color in staining; (4) absence of distortion of the architectural relations of the tissue constituents; (5) reduction of shrinkage of tissue mass and cell volume to a minimum, and (6) economy in operation.

In addition, it permits the flattening of ribbons of sections and their adhesion to glass slides by means of a formaldehyde-gelatin solution.

The technic is based primarily on the use of a true soap-wax solution of certain synthetic organic chemicals with paraffin.

From the Eugene Littauer Memorial and Fulton County Laboratories, Nathan Littauer Hospital.

1. Klebs, E.: Arch. f. mikr. Anat. 4:164, 1869.

REVIEW OF THE LITERATURE

By their affinity for and miscibility with water and paraffin, thus eliminating alcohols and clearing fluids, dioxane (diethylene dioxide), introduced by Graupner and Weissberger [2] and more recently employed by Weissberger, Young and Carleton,[3] and other similar chemicals, like butyl alcohol, have seriously threatened to break the reign of ethyl alcohol, long "the water of life" of the microscopist.

Without doubt, the most important histologic technic developed in years is the freezing-drying method of Altmann,[4] rediscovered and improved by Gersh.[5] Although the method is of great value in the work on certain histologic and histochemical problems, the complex apparatus required to freeze and dry tissues, the length of time necessary for dehydration by changing the water from the solid to the vapor phase, and the irregularity of fixation in different tissues, especially in those with a high water and lipoid content, militate, in the present stage of development of the method, against its practical use in the preparation of tissue sections.

The embedding of tissues in transparent soap masses was first practiced by Flemming.[6] Kadyi [7] infiltrated tissues with aqueous solution of sodium stearate. Osterhout [8] successfully embedded animal tissues and incompletely dehydrated plant tissues, especially delicate algae, in a coconut oil soap of his own manufacture. Orton and Post [9] experimented with a water-diffusible soap, glycol stearate (Glyco Products Company, New York), the miscibility of which with water permitted the embedding of tissues directly from aqueous mediums without preliminary dehydration. In my hands repeated trials with this material yielded inconsistent and unreliable results owing to impurities and variations in its composition.

MATERIALS

A brief consideration of the pertinent physical and chemical properties of the constituents of the soap-wax solution capable of simultaneously dehydrating and infiltrating tissues is essential for its proper application.

The chemical union of an organic base with a soap-forming fatty acid such as oleic or stearic acid produces a stearate or soap. The

2. Graupner, H., and Weissberger, A.: Zool. Anz. **96**:204, 1931.
3. Weissberger, A.; Young, J. Z., and Carleton, H. M.: Lancet **2**:1279, 1934.
4. Altmann, R.: Die Elementarorganismen und ihre Beziehungen zur den Zellen, Leipzig, Veit & Co., 1890.
5. Gersh, I.: Anat. Rec. **53**:309, 1932.
6. Flemming, W.: Arch. f. mikr. Anat. **9**:121, 1873.
7. Kadyi, H.: Zool. Anz. **2**:477, 1879.
8. Osterhout, W. J. V.: Univ. California Publ., Botany **2**:81, 1904.
9. Orton, S. T., and Post, J.: Bull. Neurol. Inst. New York **2**:302, 1932.

ethanolamines from which ethanolamine soaps are prepared are fundamentally substituted ammonia compounds in which one or more of the hydrogen atoms of the compound $N\begin{smallmatrix}H\\H\\H\end{smallmatrix}$ have been replaced by the ethanol group, $HO\text{-}CH_2\text{-}CH_2$, so that by synthesis mono-ethanolamine, diethanolamine and triethanolamine can be prepared.[10] Extensive trials indicated that mono-ethanolamine proved most suitable for union with stearic acid to produce mono-ethanolamine stearate.

Mono-Ethanolamine (Chemically Pure) (Carbon and Carbide Chemical Company).—This is a basic constituent of the soap-wax solution. It is a water-white anhydrous moderately viscous liquid with a distinct ammoniacal odor and a boiling point of 171 C. at a pressure of 757 mm. of mercury. It is one of the most highly hygroscopic substances known and is miscible in all proportions with water. From its structural formula, $\begin{smallmatrix}H\\H\end{smallmatrix}\!\!>\!N.\ CH_2CH_2OH$, it is evident that it is a base, and when dissolved in water it is strongly alkaline to phenolphthalein.

Stearic Acid.—This saturated fatty acid was selected in preference to other saturated and unsaturated fatty acids because of certain properties, especially those of a physical character. Of the many grades tested, that of the Harkness and Cowing Company, St. Bernard, Ohio, special saponified XXX stearic acid, proved most satisfactory, lacking to a large extent the oxidizing tendencies of other commercial stearic acids, so that when combined with mono-ethanolamine the stearate formed remained light colored. Prepared entirely from animal fats, it has a titer of about 55.8, a saponification value of 208, and an iodine value of 1 or under.

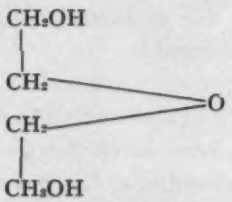
Mono-Ethanolamine Stearate.—Mono-ethanolamine, chemically pure (Carbide and Carbon Chemical Company) and saponified XXX melted stearic acid (Harkness and Cowing Company) are stirred together in molecular equivalent weights at 85 C. for from fifteen to twenty minutes by means of a high speed Hamilton Beach food mixer to form a neutral anhydrous mono-ethanolamine stearate without the evolution of water. Both chemicals must be of the highest obtainable chemical purity, for otherwise a progressive brownish darkening of the stearate by oxidation occurs at 56 C., its degree being governed by the amount of iron present as an impurity in the mono-ethanolamine and by the oxidizing tendencies of the stearic acid.

In the solid state mono-ethanolamine stearate is a stable, hard, opaque, white, waxlike substance with an agreeable soapy odor. It is water-soluble, readily taking up moisture so that it becomes opalescent and softened to a consistence comparable to that of an agar-agar gel. Like

10. Trusler, R. B.: Oil & Fat Indust. **5**:338, 1928.

many other soaps remarkable for their very large reduction in the surface tension of water by quite small concentrations, it is a first-class depressant of the surface tension of water, a property employed to advantage, as will be described under the heading of flattening of sections. It is a splendid emulsifying agent and is utilized in sufficient quantities to cause the paraffin to go into solution without separation. Its remarkable penetrating qualities promote the diffusion of the soap-wax solution into tissues under vacuum pressure.

Diethylene Glycol.—A third and important constituent of the soap-wax solution is diethylene glycol, a byproduct of the manufacture of ethylene glycol. It is a water-white, practically odorless liquid, boiling at 244.5 C., and, according to Lawrie,[11] it has the following structural formula:



In the soap-wax solution diethylene glycol is in a physical union with mono-ethanolamine stearate and paraffin. It is extremely hygroscopic and freely miscible with water, readily absorbing its own weight in nine days in a flat dish in a closed space over water (Rinkenbach[12]). Its vapor pressure under the conditions at which it is used is so low that it does not volatilize.

By virtue of its universal solvent power for dyes of most types, it accelerates their solubility, giving quicker stains and superior color values and brightness. As in the textile industry, this property is of considerable importance in the staining of tissue sections, as will be discussed under the heading of staining.

Paraffin, Melting Point 56 C. (Standard Oil Company).—This constituent is the most abundant in point of volume. Although converted into a water-soluble substance, it gives added support to tissues, being mixed with the other chemicals in the correct proportions to provide the plastic mass with the most favorable consistence for microtomy.

PREPARATION OF THE SOAP-WAX SOLUTION

A mixture is first prepared of 100 Gm. of molten stearic acid and of 400 Gm. of paraffin, melting point 56 C. This is stirred together at

11. Lawrie, J. W.: Glycerol and the Glycols, New York, The Chemical Catalogue Company, Inc., 1928, p. 385.

12. Rinkenbach, W. H.: Indust. & Engin. Chem. **19**:474, 1927.

56 C. and at high speed in a glass container by means of the Hamilton Beach food mixer. Uniformity is attained after five minutes of high speed agitation. There is now added at 85 C. a mixture of 7.5 Gm. of chemically pure mono-ethanolamine and 7.5 Gm. of diethylene glycol. Further agitation at high speed for thirty minutes yields a rich, creamy, white, air-laden material, which on being heated to drive off the air appears as a uniformly homogeneous translucent water-white solution.

The completed soap-wax solution is entirely anhydrous and "parched" and is, therefore, extremely avid for water. When melted at 65 C. it does not break down. It is perfectly stable at temperatures ranging from freezing to 100 C. and under reduced atmospheric pressures of from 400 to 800 mm. of mercury. Bacterial growth in either the melted or the solid state has never been observed, so that there is no danger of decomposition due to bacteria. Its stability in the solid state makes indefinite storage feasible.

PROPERTIES OF THE SOAP-WAX SOLUTION

The following properties were investigated: (1) odor, (2) melting point, (3) boiling range, (4) hardness, (5) hydrogen ion concentration, (6) hygroscopicity, (7) reduction of surface tension of water at 37 C., and (8) structure.

Odor.—It has a very slight paraffin-like odor at room temperature. When it is heated, the odor of mono-ethanolamine may be detected.

Melting Point.—By the capillary tube method this is 49.3 C.

Boiling Range.—This was determined by distillation in an apparatus consisting of a 125 cc. Claissen type flask, an air-cooled straight tube condenser, a graduate receiver, a Cenco vacuum pump, an open end mercury manometer and a standard laboratory mercury thermometer. The following data were obtained:

Percentage Distilled	Temperature, C.	Pressure, Mm. Hg
First drop	100	47
10	261	47
30	271	47
50	288	47
90	324	47

Thus, the boiling range is far above the temperature at which the soap-wax solution is used, viz., from 58 to 62 C.

Hardness.—Measurements of the hardness were made with an asphalt penetrometer at 25 C. A standard asphalt needle was used with a 200 Gm. load. The time of penetration was five seconds. The results indicated in the accompanying tabulation were obtained on the soap-wax solution in the solid state, as well as on other materials for com-

parison, the larger hardness numbers indicating the softer materials, since the numbers indicate the depth of penetration of the needle.

Material	Hardness
Soap-wax	38
Paraffin (Standard Oil Company) (melting point 56 C.)	21
Mono-ethanolamine stearate	13
Stearic acid (Harkness and Cowing Company, special XXX)	7

The data indicate that the solidified soap-wax solution is relatively softer than the usual paraffin melting point 56 C., employed for embedding, and, together with the absence of crystallinity, discussed later under the heading of structure, this accounts in large part for the greater ease in the microtomy of tissues prepared with it in comparison with tissues embedded in paraffin.

Hydrogen Ion Concentration.—This is p_H 7.5 at 40 C. (Beckman p_H meter).

Hygroscopicity.—Comparative determinations of hygroscopicity were made with the entire soap-wax solid, mono-ethanolamine stearate and diethylene glycol by measuring the amount of water vapor absorbed in a saturated atmosphere at temperatures varying from 25 to 32 C. over a period of several days. This was done by placing approximately 5 Gm. samples of the material in covered round tin-plated iron dishes ⅝ inch (1.59 cm.) deep and 1 15/16 inch (4.92 cm.) in diameter. The solids were first carefully melted in the containers to provide the same exposed surface area. After being weighed, the samples were placed in a humidity cabinet in which forced circulation of water-saturated air was maintained. Weighings were made at intervals and the percental gain in weight calculated as follows:

	Percental Gain in Weight		
Time, Hours	Soap-Wax	Mono-Ethanolamine Stearate	Diethylene Glycol
24	0.05	0.22	39.2
72	0.24	0.58	89.4
96	0.34	0.79	150.0

It is clear from these data that the burden of dehydration falls chiefly on diethylene glycol.

Reduction of Surface Tension of Water.—When mixed with water the soap-wax solid forms emulsions which are quite stable at ordinary temperatures in concentrations of approximately 15 per cent by weight or over. The stable emulsions are pastes. At lower concentrations separation occurs and a waxy solid floats on the surface. Measurements of surface tension were attempted at 37 C. with the du Nuoy apparatus but with no success. Measurements were made on dilute solutions, but the wax floating on the surface interfered, and the results,

while indicating the depression of surface tension, are probably not very accurate. The following data were obtained by heating dilute mixtures to 50 C., allowing them to cool slowly, and measuring the surface tension at 37 C.

Soap-Wax in Mixture by Weight, per Cent	Surface Tension, Dynes/Cm/.	Temperature, C.
0.125	34.0	37
0.5	34.5	37
1.0	38.2	37
8.0	44.4	27

Structure.—Ultramicroscopic examination of the molten soap-wax preparation reveals no discrete liquid particles dispersed in a liquid medium. Care was taken to bear in mind that with identical indexes of refraction of the dispersed phase and dispersion medium an emulsion would appear homogeneous. Therefore, it can be concluded that the soap-wax preparation is a true solution and not an emulsion. Another fact which supports this conclusion is the increase of solubility of the various components in each other with increase in temperature.

An x-ray spectrogram reveals no evidence of crystallinity. The film shows continuous blackening instead of the series of diffraction lines which would have been obtained had the soap-wax solution contained crystals more than a few hundred molecules across. The method used is that of von Laue and of Bragg,[13] wherein the three dimensional atomic lattice of crystal structure serves as a diffraction grating for very short x-rays. The apparatus used was the General Electric Company's standard x-ray diffraction apparatus with a molybdenum target tube, zirconium dioxide filter, fluoroazure intensifying screens and the Eastman Kodak Company's superspeed contrast duplitized film. The time of exposure was eight hours; the voltage across the tube was 30 kilovolts; the tube current was 30 milliamperes; the radius of the film was 8 inches (20.32 cm.).

GENERAL CONSIDERATIONS OF METHOD

The principles on which the technic is based are simple. After preliminary extraction of the tissue fats and partial removal of the fixative for sixty minutes with acetone, the tissue blocks are simultaneously dehydrated and infiltrated in vacuo at from 58 to 62 C. for one hour in a specially devised drying vacuum apparatus,[14] and are then ready to be placed in the molds and cut by microtome.

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13. Bragg, William H.: Introduction to Crystal Analysis, New York, D. Van Nostrand Company, Inc., 1929. Davey, W. P.: A Study of Crystal Structure and Its Applications, New York, McGraw-Hill Book Company, 1933.

14. Dushman, S.: The Production and Measurement of High Vacuum, Schenectady, N. Y., General Electric Co., 1922.

DESCRIPTION OF APPARATUS

The apparatus used in the simultaneous dehydration and infiltration technic is shown in figure 1. I am indebted to Dr. Saul Dushman and to Mr. F. A. Opps for help in the development of the oven.

The vacuum is created by a Cenco Hyvac pump (*A*) actuated by ⅙ horsepower General Electric motor (*B*). The unit is entirely concealed in the interior of the wooden cabinet but is still readily accessible

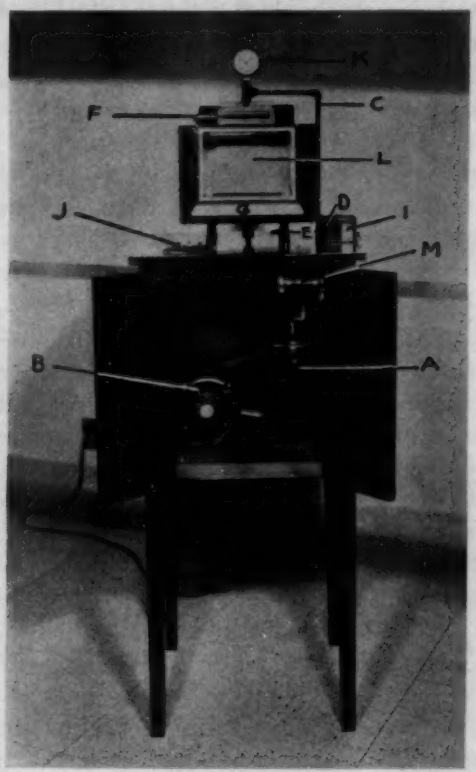

Fig. 1.—Vacuum drying oven, with cabinet doors open to show the motor and pump. *A* indicates the pump creating the vacuum; *B*, the motor which drives it; *C*, pipe-line to the vacuum chamber; *D*, the vacuum valve; *E*, the air valve; *F*, windows for observation and illumination; *G*, the rubber-edged door of the vacuum chamber; *I*, the thermostat control box; *J*, electric controls; *K*, a gage; *L*, the vacuum chamber; *M*, outlet.

for oiling and adjustment. The pump is connected by a steel pipe-line (*C*) to the vacuum chamber (*L*), described in the next paragraph. This pipe-line runs from the vacuum chamber to a three way T outlet (*M*), one outlet leading to the pump, the second to the vacuum valve (*D*), and the third to the air valve (*E*). These needle valves, one

on the operator's right for slow exhaustion of the inner chamber and the other on the left for slow admission of air to break the vacuum, are conveniently placed on the top of the wooden cabinet.

The vacuum chamber consists of an outer protective steel jacket, lacquered in black, and an inner working chamber. The protective jacket is lined internally with a 2 inch (5.08 cm.) thickness of asbestos wool packing, except at the observation and illumination windows (*F*). This packing prevents outward radiation of heat. The over-all dimensions of the steel jacket are 11 by 8.5 by 11.5 inches (27.94 by 21.59 by 29.21 cm.).

The angularity of the jacket top permits directing the light from a small 100 watt General Electric gas-filled bulb into the inner chamber for convenient observation of the interior without glare from the light source. The glass of the observation and light windows is pyrex, capable of resisting atmospheric pressure when the vacuum pressure is 400 mm. or higher and the temperature is 60 C. or over. The glass is held between air-tight rubber seals to allow for expansion and contraction due to changes in temperature.

The rubber-edged door (*G*) of the vacuum chamber is constructed of duralumin, strengthened by two braces of the same metal to distribute uniformly the force of the locking levers which seal the door against the interior of the chamber. Elongated openings on the door hinges allow for compression of the inner rubber seals to insure proper closure. A door stop holds the door open in the horizontal position so that the flat surface forms a convenient tray. When securely closed by the locking levers, the door completes the air-tight vacuum chamber.

Three heating elements of the General Electric commercial strip-heater type are applied against the outer wall of the vacuum chamber, on the bottom and on both sides, replaceable in the event of a burn-out. These heating elements are wired to an automatic General Electric thermostat control box (*I*) placed on the top of the cabinet, with the sensitive mercury bulb applied against the floor of the inner chamber, ½ inch (1.27 cm.) from the heating element. This thermostat maintains a constant temperature within the vacuum chamber with a tolerance of ± 1 C. Approximately three quarters of an hour is required to bring the vacuum chamber to a working temperature of 60 C., but in practice the heating elements remain in operation continuously, as in an incubator.

The electric controls (*J*) operating the light bulb, the heating elements and the pump are situated on the top of the cabinet.

The gage (*K*) indicating the level of vacuum pressure is a United States Gauge Company dial 2½ inches (6.4 cm.) in diameter, graduated

to 750 mm. vacuum, each graduation denoting 10 mm. It has a technical tolerance of error of 2 mm. vacuum.

The vacuum chamber (*L*), with a working space 6 inches (15.24 cm.) high, 8 inches (20.32 cm.) wide and 8 inches deep, is constructed of rolled sheet duralumin 5/16 inch (0.79 cm.) in thickness to withstand atmospheric pressure, fabricated by welding it into one piece to prevent the entrance of air. Duralumin was selected because of its good conductivity of heat and resistance to erosion by the soap-wax solution. Raised slightly from the floor of the cabinet is an asbestos tile platform, ¼ inch (0.64 cm.) in thickness, which tends to maintain more uniformly the temperature of the containers. During treatment of as many as twenty-four to thirty blocks of tissue in the soap-wax solution under vacuum the apparatus requires no personal attention.

Although vapor pressure is produced within the vacuum chamber by the withdrawal of water from the tissue blocks, there is a fall of only 30 to 40 mm. vacuum within one hour during the simultaneous dehydration and infiltration of as many as twenty-four blocks. This water of dehydration passes through the oil which seals the Hyvac pump and escapes through an opening on top of the pump to the outside after the vacuum within the chamber is broken by the admission of air, thus preventing contamination of the oil in the vacuum pump with condensed water.

The blocks of tissue are placed in small glass containers on nickel wire mesh (no. 20), which is resistant to the action of the soap-wax solution. The containers are of clear glass; the largest measures 80 mm. and the smallest 36 mm. in diameter. The series of five containers will accommodate, respectively, 1, 4, 6, 8 and 16 blocks of tissue approximately 1.5 cm. square. The edge of each nickel mesh is turned down so that the tissue blocks are raised 0.5 cm. from the bottom of the container, insuring their complete immersion in the dehydrating and infiltrating medium. Eighty blocks of tissue can be accommodated at one time in the vacuum chamber.

APPLICATION OF TECHNIC

In the development of the technic, consideration was taken of the thickness, surface area and number of blocks to be simultaneously dehydrated and infiltrated, as well as of their differences in structure, chemical composition and the presence of a limiting capsule. In spite of these differences, all tissues regardless of their origin, except breast and pure fat, undergo the same treatment.

For optimal results, the blocks should be from 2 to 3 mm. thick and 140 mm. or less square. Objects of solid tissue may be readily cut at a uniform thickness of 2 or 3 microns, without wedging or crushing,

with a modified double-edged Valentin knife.[15] This knife is unsuccessful with friable or delicately textured tissues, such as lung.

1. *Fixation.*—The fixation of tissues is important. Successful simultaneous dehydration and infiltration is dependent on successful fixation. The relative firmness produced by different fixatives is important, for the softer and less shrunken the tissue the better the final preparation.

The fixative par excellence is neutral 10 per cent solution of formaldehyde, although Zenker's solution and Martin Heidenhain's [16] Susa mixture are entirely satisfactory. Prolonged fixation in solution of formaldehye, notorious for its hardening effect and interference with hematoxylin staining, does not render sectioning of soap-treated tissues unduly difficult, while staining, owing to the presence of diethylene glycol, is excellent. Successful preparations of tissues of the central nervous system for topical studies can be obtained without difficulty. Bouin's fluid [17] or other fixatives containing trinitrophenol are counterindicated in that the formed tissue albuminous salts of trinitrophenol for some still undetermined reason interfere with the diffusion of the soap-wax solution.

Tissues fixed in solution of formaldehyde and the Susa mixture need not be washed in running water. Thus, the formaldehyde gas dissolved in water is partly extracted by acetone and is then readily evaporated in vacuo from tissues. As usual, tissues fixed in Zenker's solution should be washed over night in running tap water to remove the excess of potassium bichromate.

2. *Preliminary Extraction of Fat with Acetone.*—The inability of the molten soap-wax solution to diffuse into tissues in the presence of fats and lipoids, constantly present in variable amounts, requires their removal by acetone, an energetic fat solvent. Unfortunately, repeated attempts to incorporate into the soap-wax solution this fat solvent or one more efficient than acetone, such as ethylene dichloride or dichlorethyl ether, failed owing to their volatility in vacuo at 60 C. The incidental extraction of water from tissue by acetone is relatively small, amounting approximately to only from 15 to 20 per cent of the total.

One should allow 5 cc. of acetone for each block. For optimal results all tissues except breast and pure fat are immersed in acetone for one hour with one change after thirty minutes. Pure fat and breast require two hours with a change after one hour and shaking from time to time. If desired, the acetone can de dispensed with in

15. Ranvier, L.: Traité technique d'histologie, Paris, F. Savy, 1875.

16. Heidenhain, M.: Ztschr. f. wissensch. Mikr. **33**:232, 1916-1917.

17. The formula is: Solution of formaldehyde U. S. P., 500 cc.; distilled water, 1,500 cc.; glacial acetic acid, 100 cc.; trinitrophenol to saturation point.

the preliminary treatment of lung. Tissues fixed in dilute solution of formaldehyde U. S. P. and alcohol or in absolute alcohol for glycogen do not require treatment with acetone, as the alcohol serves to extract the fats.

3. *Simultaneous Dehydration and Infiltration in Molten Soap-Wax Solution at from 58 to 62 C. and from 350 to 400 Mm. Vacuum.*—Infiltration of tissue in vacuo, although introduced by Hoffman in 1884, has not been widely practiced in spite of the obvious advantages of speed and efficiency. The treatment of blocks with molten soap-wax solution in vacuo eliminates troublesome gas and air bubbles situated in the embedding matrix near the face of the object. It insures uniform and complete dehydration and infiltration of tissues varying in consistence, without injury to the finer organic structure or collapse of internal cavities.

After the preliminary extraction of tissue fats and lipoids by acetone, the blocks are transferred directly to the glass containers and placed on nickel mesh screens. A volume of 5 cc. of the soap-wax solution, previously melted in a water bath, is used for each block of 2 or 3 mm. thickness and 150 mm. or less square. Although the solution has a melting point of only 49.3 C., yet it should be kept at from 58 to 62 C., for with an increase in temperature there is a reduction in viscosity, thus aiding diffusion. The containers are placed in the vacuum chamber of the drying oven, which is then hermetically sealed by tightly turning the locking levers.

After the door is secured, both valves are closed by turning them to the right as far as possible. The motor is then started by the third switch on the control panel. The exhaust valve on the right is opened to produce at slow speed a low pressure in the chamber, from five to ten minutes being allowed for the reduction of the atmospheric pressure to 400 mm. vacuum as registered on the gage dial. When the 400 mm. mark is reached, the exhaust valve is tightly closed by turning it to the left, the motor is stopped, and the air valve on the left is opened wide. During the treatment of from twenty-four to thirty blocks of tissue in the soap-wax solution under vacuum for one hour the apparatus requires no personal attention.

At the end of the run, viz., in from forty to fifty minutes, the vacuum is broken by turning the exhaust valve to the left enough to allow a slow return to atmospheric pressure in from five to ten minutes. When the apparatus is not in use, both valves must remain open to prevent oil being drawn from the pump into the pipe-line. The objects are now ready to be embedded in the mold.

If exhaustion or admission of air is carried out too rapidly, the alveolar walls of pulmonary tissue are ruptured with the production of

artefacts, and the epithelial cell cords of organs such as the adrenal gland and liver are not only widely separated from each other but disintegrated as well.

The presence of water in the tissues and its necessary withdrawal, together with paraffin infiltration at 58 C. and the shrinkage caused by the clearing fluids and fixatives such as Zenker's solution and the Susa mixture, naturally lead to shrinkage no matter how cautiously dehydration and infiltration are practiced. Unfortunately, there is no precise method for the determination of shrinkage. Careful consideration was given to the methods of Donaldson,[18] Tarkhan,[19] Hertwig [20] and others with their advantages and limitations, but, although applicable in estimating the influence of fixation on tissue masses and volume, they cannot be successfully applied to indicate the degree of shrinkage after treatment with the soap-wax solution. However, comparative examination of sections treated with the same fixative but with different methods of dehydration, clearing and infiltration shows obvious differences (fig. 2). The lesser degree of shrinkage after the use of the soap-wax solution is explained on the basis of the elimination of the shrinking action of absolute alcohol and of the usual clearing reagents and on that of the reduction of the exposure to heat from three hours to one hour.

4. *Embedding.*—Embedding is carried out at room temperature in Leuckhart embedding boxes resting on a glass plate. The blocks of tissue are rapidly passed through a Bunsen flame to melt any excess of wax-soap solution. Orientation of the objects is effected by means of a warmed forceps. More than one block of tissue may be embedded in different parts of the mold provided that each object is enclosed in the solidified soap-wax on parallel sides to a thickness of at least from 5 to 6 mm.

Freshly molten soap-wax solution heated to 65 C. is poured over the objects. Sudden immersion in cold water to prevent crystal growth, as is usually practiced with paraffin, is unnecessary since the medium is noncrystalline. The blocks are allowed to cool at room temperature for from ten to fifteen minutes and are then chilled in the icebox for from thirty to forty-five minutes. Excessive chilling leads to cracking of the soap-wax matrix in tissues from the nervous system, thereby militating against optimal microtomy. Brain blocks should be solidified at room temperature for from twenty to thirty minutes.

The resultant mass is opaque white, homogeneous and free from cavities. The blocks are readily removed from the lead boxes. They

18. Donaldson, H. H.: J. Morphol. **9**:123, 1894.
19. Tarkhan, A. A.: J. Roy. Micr. Soc. **51**:387, 1931.
20. Hertwig, G.: Ztschr. f. mikr.-anat. Forsch. **23**:484, 1930.

keep indefinitely provided that the tissue face of the object is hermetically sealed by a thin coat of paraffin to prevent drying out. Embedded tissues thus prepared keep for three months or more and may be cut by microtome as well as when first embedded.

The plastic soap-wax material surrounds not only the entire object but each separate cell, thus forming within the embedded tissue a composite body.

5. *Sectioning.*—The cutting properties of the soap-wax mass are definitely superior to those of paraffin, pyroxylin (celloidin) or pyroxylin and paraffin. This superiority is due to its noncrystallinity and higher hardness number, also to the elimination of the hardening and shrinking action of the higher alcohols, the dealcoholizing fluids and the repeated prolonged hot paraffin baths. In addition, there is less wear and tear on the cutting edge of the knife, which requires less frequent sharpening.

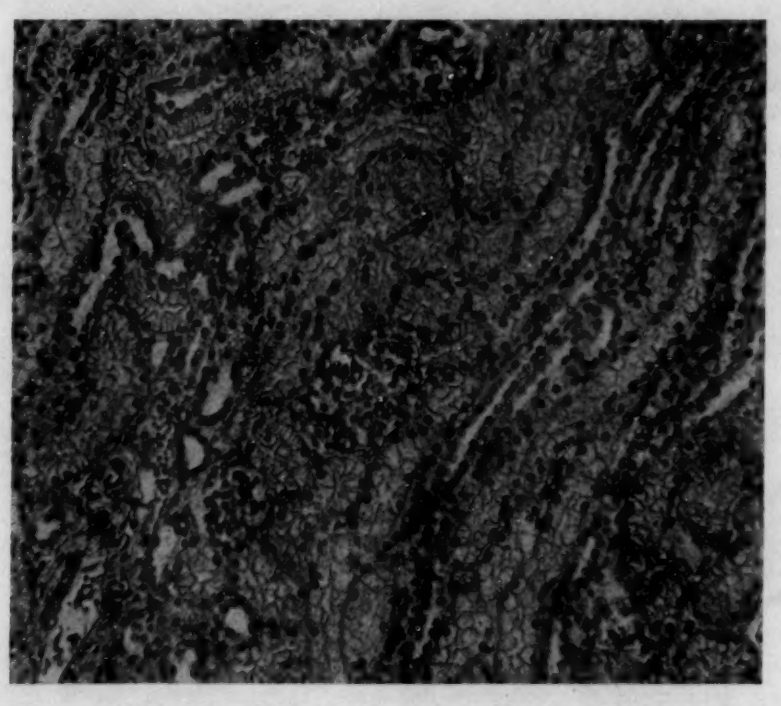

Fig. 2.—Kidney of a child aged 22 months. The tissue block was fixed in Susa fluid for two hours. It was cut with a double-edged knife. Soap-wax sections were made and stained with Harris' hematoxylin and erythrosin stain. Note the reduction of shrinkage in the glomerulus with absence of subcapsular space, the small lumens of the convoluted tubules, the presence of lumens in the collecting tubules and loops of Henle and the clarity of cell detail. The section is 5 microns thick; × 225.

After being trimmed, the soap-wax block with its embedded object is firmly clamped in the holder of the microtome and properly angled to the knife. With the large model Spencer rotary microtome adjusted at from 2 to 6 microns, the normal range for general routine and cytologic work, the individual sections adhere so as to form excellent ribbons, less compressed and presenting fewer pleats than those of paraffin. They are uniform in thickness and free from tearing, splitting, cracks or breaks. Tissue objects differing markedly in density yield satisfactory ribbons. The preparation of the face of originally brittle tissue blocks with collodion is unnecessary. Serial sections are readily cut.

Although compressed, pleated ribbons are secured with the large model Spencer rotary microtome set at 1 micron, it is highly questionable whether sections of this thickness are actually obtained. Kisser [21] stated that sections of this thickness probably will never be obtained because of certain limiting factors, viz. (1) the presence of teeth and notches on the cutting edges of excellently sharpened knives examined microscopically at a magnification of more than 100 diameters and, even more important, (2) the progressive curling and undulation of the knife edge with increasing thickness of the section and the vibration of the edge at the moment of impact of the knife on the object. In point of fact, the cutting edge is actually more than 1 micron in thickness and would cause wedging rather than clean and sharp cutting of the tissue sections at 1 micron or less.

6. *The Flattening of Sections and Their Adhesion to Glass Slides.*—The flattening of the ribbons of sections of tissues treated with soap-wax solution and their adhesion to glass slides are secured by means of a formaldehyde-gelatin bath. This development of the technic was necessitated because of (1) the relative or complete inefficiency for various reasons of a large number of adhesives, including Mayer's glycerin-egg albumin and sodium silicate, and (2) the sharp reduction of the surface tension of the water in the water bath employed for flattening or of the film of water on the glass slide in the water and albumin method by the stearate in the soap-wax solution with consequent dissolution of the tissue sections and of the soap-wax framing them. Like other soaps, mono-ethanolamine stearate is an excellent depressant of the surface tension of water.[22]

The rationale of the technic is simple. Quincke [23] has shown that gelatin with a specific gravity of 1.02 reduces the surface tension of

21. Kisser, J.: Naturwissenschaften **16**:223, 1928.

22. Lawrence, A. C. S.: Soap Films: A Study of Molecular Individuality, New York, Industrial Book Co., Inc., 1929.

23. Quincke, G.: Ann. Physik. **10**:507, 1903.

water against air 7.272 dynes per centimeter, as compared with a reduction of 2.672 dynes per centimeter produced by 0.0025 per cent Venetian soap and a reduction of 8.253 dynes per centimeter produced by water. In accordance with Gibbs' law of surface concentration,[24] gelatin in a solution tends to be more highly concentrated at the surface than at other layers. By virtue of this concentration and of its relatively slight reduction of surface tension, a 1 per cent formaldehyde-gelatin bath admirably flattens out ribbons of sections by dispersion after a few seconds without mechanical manipulation or injury and without leaving folds or wrinkles. When completely desiccated on the hot plate, formaldehyde-gelatin serves as an adhesive far more secure than glycerin-egg albumin in that it is unaffected by the weak alkaline solutions used to "blue" sections or by any other reagents or stains used in the staining process.

The gelatin bath is prepared as follows: 10 Gm. of Milligan and Higgins Corp. no. 850,669 granulated gelatin[25] is allowed to swell in 150 cc. of distilled water for from twenty to thirty minutes in the icebox; to it is added with stirring 850 cc. of distilled water at from 60 to 80 C. and then 10 cc. of 37 per cent neutral (white label) formaldehyde (Merck). The gelatin is coagulated by the formaldehyde with the formation of a condensation product, the exact chemical composition of which is unknown. The resultant solution is slightly opalescent and free from floating particles. It is indefinitely stable, but, owing to evaporation in the constant temperature bath with reduction in volume and alteration in the concentration of the gelatin after its use for one week, a fresh solution must be prepared weekly. Because the formaldehyde-gelatin solution gels at room temperature, when ready to be used it is warmed slowly to from 37 to 41 C. to preserve its gram strength and to bring it into solution.

Slides are cleaned by freeing them from grease by immersion for a few minutes in equal parts of acetone and 95 per cent alcohol and are then dried by exposure to air. Removal of the grease is essential in that

24. Thomas, A. W.: Colloid Chemistry, New York, McGraw-Hill Book Company, 1932, p. 242.

25. Mr. Carl Ackerbauer, research chemist of the Milligan and Higgins Corp., Johnstown, N. Y., made it possible for me to use this gelatin and gave valuable advice. It was selected in preference to other "blended" and "straight" gelatins because of its p_H, solubility in water, particle size and extremely high gram or tensile strength, all of which are important in the reduction of surface tension and in the adhesion of sections to slides. Not least in importance is its availability.

it not only reduces the tensile strength of the formaldehyde-gelatin solution but also prevents the penetration of and filling in of surface pores of the glass slides—the secret of good adhesion.

The formaldehyde-gelatin solution warmed to from 39 to 40 C. is poured into the pan of a constant temperature bath controlled by a General Electric thermostat.

The constant temperature bath consists of a wooden platform, lacquered in black and mounted on four short legs, supporting an immovable steel jacket, which houses the heating element and which is hollow in its center to hold an aluminum tray in which tissue sections are floated on the gelatin solution. A General Electric thermostat control is also mounted on the wooden platform, maintaining the working temperature of from 37 to 41 C. with a total deviation of ± 1 C. An off-on switch and plug unit complete the apparatus. The over-all dimensions of the bath are 17.5 by 9.5 by 6.5 inches (44.45 by 24.13 by 16.51 cm.) and of the tray 9.5 by 2.5 by 5.75 inches (24.13 by 6.35 by 14.61 cm.).

The temperature should not be allowed to pass beyond 41 C. as the surface tension is reduced. The ribbon is placed on the surface of the gelatin bath, and after a few seconds it flattens out without manipulation except for very coarse folds. It is handled with ease. A strip of two or three sections is cut off of the ribbon and transferred on a glass slide to a water bath at from 39 to 42 C. It is left in this bath for from ten to fifteen seconds to dissolve the excess of gelatin adherent to the undersurface of the sections. From time to time, the film of gelatin floating on the surface of the water bath is skimmed off with a strip of absorbent paper. The section is now floated on to a fresh slide, which is allowed to drain for a few seconds. The remaining gelatin over its back and about the section is carefully removed with a cloth. Without blotting, the slide is desiccated to dryness on the hot plate at from 40 to 45 C. for thirty minutes, thus eliminating the interposition of a layer of air between the slide and section, which would prevent good adhesion while drying. Exposure of the sections to cold air currents leading to premature gelation of the gelatin and production of folds should be avoided. The sections are now ready to be stained.

The formaldehyde-gelatin adhesive does not interfere with staining. Except for a small amount of stained aggregates of gelatin particles about the section, the slide is clear. There is no retraction of the collagen during desiccation which would lead to irregular staining. The danger of adventitious bacteria in sections, so important in stains for bacteria in tissues, is avoided by the action of formaldehyde on the gelatin. Sections can be stained in heated solutions without detachment.

STAINING

When one is ready to stain, the soap-wax is removed from the slides by immersion for ten minutes in chemically pure carbon tetrachloride, followed by one change in 95 per cent alcohol for from three to five minutes and then two changes in tap water for a few minutes each, from which they are transferred as usual to the staining reagents. Tissues fixed in mercuric chloride or in any fixative containing this salt should be treated as usual with alcoholic iodine solution and hyposulfite until the natural color of the sections has reappeared.

As stated before, the presence of diethylene glycol in the sections accelerates the staining with common aniline dyes and, in addition, produces superior definition of color. Although diethylene glycol is a dye solvent for such natural organic dyes as hematoxylin and brazilin, its concentration in the soap-wax solution is too low to shorten the usual staining times of these dyes, but definition equal to that of paraffin sections is obtained. Nevertheless, as would be anticipated, the precision and clearness of the stained microscopic images in soap-wax sections are also influenced by the sensitiveness of the dye to preliminary fixation.

With common aniline dyes, the regular staining time is reduced from one-third to one-half, and even more with certain counterstains such as 1 per cent aqueous erythrosin (Grubler) and Van Gieson's solution. A suitable colorimeter for the objective determination of the definition of color in stained histologic sections in terms of wavelength, intensity and saturation rather than as psychologic attributes [26] was not available. The usual methods of determining the definition of color—the use of a comparison microscope, color charts and camera lucida devices—proved inaccurate and unreliable because the eye is not an analytic instrument. However, it is obvious even from observation with the naked eye that the colors produced in soap-wax sections, especially those by synthetic aniline dyes, are delicate, precise, and of a greater intensity and brilliance in comparison with those stained after the usual methods of dehydration and infiltration, especially after fixation in Zenker's solution and the Susa mixture.

Typical Micro-Anatomic Stains.—1. Hematoxylin Stain: Soap-wax sections stained with Delafield's or Harris' hematoxylin and counterstained with either aqueous eosin or erythrosin are satisfactory. As is well known, formaldehyde inhibits to a limited extent staining with eosin. On the other hand, the eosin color in formaldehyde-fixed soap-wax sections tends to be purer and deeper in hue. Regardless of the type of preliminary fixation, Van Gieson's solution of trinitrophenol

26. Hardy, A. C.: A Handbook of Colorimetry, Cambridge, Mass., Massachusetts Institute of Technology, 1936, p. 12.

and acid fuchsin can be successfully applied after primary staining with Delafield's or Harris' hematoxylin or Weigert's iron-hematoxylin. Special care must be taken not to overstain in the solution of trinitrophenol and acid fuchsin as the diethylene glycol promotes the development of the colors within sixty seconds or less. The best results with Van Gieson's solution are obtained after fixation in Zenker's solution.

2. Eosin and Methylene Blue Stain:[27] This staining method furnishes excellent results, especially after fixation in Zenker's solution or Martin Heidenhain's Susa fluid. It is recommended that from 0.25 to 0.5 per cent colophony be added to 95 per cent alcohol, as advised by Mallory and Wright to permit of sharper differentiation.

3. Mallory's Aniline Blue-Acid Fuchsin-Orange G Stain for Connective Tissue:[28] The collagen in soap-wax sections, especially after fixation in Zenker's solution or Susa fluid, is colored a strikingly bright blue and is sharply defined, together with the structure of the cells and their pathologic products, such as mucin and amyloid.

4. Masson's Trichrome Stains:[29] These triple stains furnish exquisite stained microscopic images in soap-wax sections after fixation in bichromate-formaldehyde solution, Zenker's solution or Susa fluid with hemalum-erythrosin-saffron, iron-hematoxylin-ponceau-acid fuchsin-aniline blue, iron hematoxylin-ponceau-acid fuchsin-light green, and the iron-hematoxylin-acid fuchsin-yellow metanil methods. For Masson's trichrome stains the soap-wax sections are prepared as usual, but collodionization to reduce the discoloration of the saffron by alcohol is omitted. Euparol may be substituted for Canada balsam, and the sections permanently mounted in this medium directly from xylene. Not only does euparol provide a better index of refraction for the visibility of delicate cell structure than neutral balsam, but it also permanently preserves the tints.

5. Weigert's Elastin and Van Gieson Stain:[30] This useful combination for the demonstration of both elastic and collagen fibers, using phenol-basic-fuchsin instead of resorcin-fuchsin, can be successfully applied to soap-wax sections. Hart's modification of Weigert's elastic tissue stain also furnishes brilliant images of the elastic fibrils and can be counterstained with Van Gieson's solution after fixation in formaldehyde solution, Zenker's solution or Susa fluid.

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27. Mallory, F. B., and Wright, J. H.: Pathological Technic, Philadelphia, W. B. Saunders Company, 1918, p. 94.

28. Mallory and Wright, cited by Masson,[29] p. 111.

29. Masson, M. P.: Tumeurs diagnostics histologiques, Paris, A. Maloine & Fils, 1923, p. 684.

30. Carleton, H. M.: Histological Technique, London, Oxford University Press, 1926, p. 131.

Typical Cytologic Staining Methods.—1. Heidenhain's Iron-Hematoxylin Stain: Excellent stained images are obtained with the iron-alum-hematoxylin of Heidenhain, perhaps the most important of all cytologic stains because of its precision. The staining time of the hematoxylin is not shortened, but the definition of color and the preservation of the finer details of the cells and the optical qualities of the colored cell structures are excellent. The duration of the iron-alum mordant and hematoxylin baths, as in the case of paraffin sections, is almost entirely dependent on the fixative employed. Almost any counterstain may be used with success.

2. Unna's Carbolpyronine-Methyl Green Stain [31]: Unna's modification of Pappenheim's method for the demonstration of the oxyphilic or basophilic character of cell constituents can be successfully applied after Susa fixation, the cytoplasm of the plasma cells appearing bright red.

3. Cajal's Uranium-Formaldehyde Method for the Internal Reticular Apparatus of Golgi [32]: After reduction of the pieces that have been impregnated with silver nitrate these are washed quickly, allowed to remain in the acetone for thirty minutes, and then simultaneously dehydrated and infiltrated in the soap-wax solution in vacuo for one hour.[33] For the original Cajal fixative, Da Fano's cobalt nitrate modification may be substituted to advantage.

Staining Methods for Bacteria in Sectioned Material.—Giemsa's method for staining bacteria in tissues, the Brown and Brenn [34] method for gram-positive and gram-negative bacteria, the Gram-Weigert technic for gram-positive bacteria and the Ziehl-Neelsen carbolfuchsin staining method for acid-fast bacteria can all be successfully applied to sections of material subjected to the soap-wax technic.

Staining Methods for Leukocytes in Tissues.—The soap-wax method is well adapted for the study of leukocytes in that there is less shrinkage combined with good preservation of the delicate structures of the cells. The azure II-eosin and hematoxylin method of Maximow [35] after fixation in Helly's fluid stains differentially and brilliantly the leukocyte granules, the nuclear chromatin appearing dark blue and the nucleoli purple. Excellent stained images are also obtained with Dominici's valuable trichrome stain—erythrosin-orange G-aqueous toluidine blue.[36]

Neurologic Methods.—1. Stains for Normal Myelin Sheaths: The Weigert method and its modification by Pal are inapplicable to soap-wax-

31. Carleton,[30] p. 145.
32. McClung, C. E.: Microscopical Technique, New York, Paul B. Hoeber, Inc., 1929, p. 336.
33. McClung,[32], p. 338.
34. Brown, J. H., and Brenn, L.: Bull. Johns Hopkins Hosp. **48**:69, 1931.
35. McClung,[32] p. 263.
36. Masson,[29] p. 694.

treated tissue because of irregular staining and difficulty in differentiation. However, the Heidenhain iron-hematoxylin stain applied to formaldehyde-fixed tissue according to the technic of Morgan [37] yields results equal to those of Weigert. For consistently optimal results the sections should be cut 10 microns in thickness and preferably mordanted for from two to three hours in 4 per cent aqueous solution of iron-alum, stained for from eight to twelve hours in hematoxylin, and differentiated in 2 per cent iron-alum for a few minutes until the larger fiber tracts appear sharply outlined. The intensity of the stain is not altered by varying the duration of the mordanting. The differentiation is completed in 0.5 per cent hydrochloric acid for a few seconds, the background appearing almost colorless and the fiber tracts well differentiated. The success of the stain depends almost entirely on its accurate differentiation, which should be controlled under the microscope. The stain is permanent provided that the iron-alum is completely washed out in running water after differentiation. Practically any counterstain, including Morgan's neutral red ripened by colon bacillus, may be employed for the nerve cells. Successful preparations of test material, such as the supraradial feltwork of the human cerebral cortex, show intense dark blue coloration of the myelinated fibers. The method eliminates the uneven staining, difficulties in differentiation, loss of selective cell staining, brittleness of the specimen and the tediousness due to mordanting associated with the older technics. The entire staining process requires but a few hours and is applicable to old as well as to fresh material following the use of nearly any fixative, with excellent results.

The Alzheimer-Mann method [38] of staining for glia cells with an eosin-methyl blue mixture can also be employed to demonstrate myelin sheaths and axis-cylinders. After fixation of the blocks in Weigert's *Gliabeize* and dilute solution of formaldehyde U. S. P., sections 5 microns in thickness are cut, and after preliminary mordanting these are stained in Mann's solution. Since the eosin and methyl blue are synthetic dyes, the duration of staining is shorter than usual. The method, valuable because it enables one to stain selectively degenerated axis-cylinders within a few days after the onset of injury, gives splendid pictures, characterized by the brightness of the colored myelin sheaths, axis-cylinders, glia cells and their fibers.

2. Mallory's Method for Neuroglia: In comparison with paraffin sections prepared according to the usual methods of dehydration and infiltration, Mallory's phosphotungstic acid-hematoxylin method for neuroglia fibrils is shorter in duration in the hematoxylin bath and yields as well

37. Morgan, L. O.: Anat. Rec. **32**:283, 1926.

38. McClung,[32] p. 402.

more brilliantly stained images of the fibrils. Fixation in Zenker's solution is recommended, although the method is also applicable to tissue fixed in solution of formaldehyde or in this plus saline solution. The outline of the fibers is not quite as sharp as after fixation in Zenker's solution or Susa fluid.

The molybdic acid-hematoxylin method of Held [39] for the demonstration of marginal glia, and the victoria blue method of Anglade and Morel [40] for neuroglia fibers may also be employed with satisfaction.

3. The Bielschowsky-Agduhr Method for Neurofibrils [41]: Excellent results are obtained with the application of this method to sections from

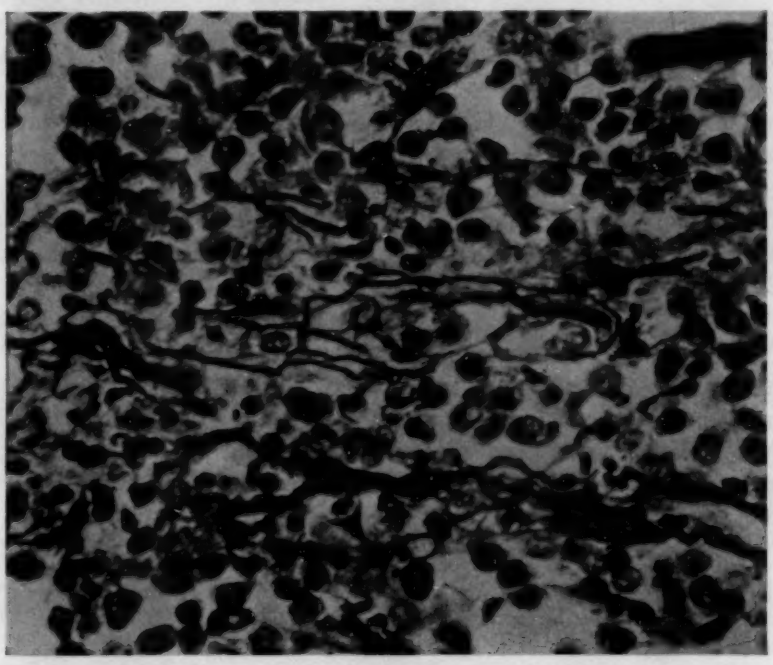

Fig. 3.—Lymphosarcoma of the rectum. The tissue block was fixed in solution of formaldehyde, embedded in soap-wax and sectioned at 5 microns. Note the silver impregnation of the reticulum fibers according to the technic of Wilder; × 725.

5 to 6 microns in thickness. In point of time, the wax-soap sections offer no advantage. Successful preparations show that the finer extracellular fibrillae are selectively impregnated, and that the coarser dendrites,

39. Lee, A. B.: The Microtomist's Vade Mecum, ed. 8, Philadelphia, P. Blakiston's Son & Co., 1921, p. 484.

40. Carleton,[30] p. 266.

41. Bielschowsky, M., and Agduhr: Ztschr. f. wissensch. Mikr. **34**:1, 1917.

axons and neurofibrils present a moderately deeper black color against a yellowish gray background than is found in corresponding paraffin sections. The bodies of the nerve cells are not excessively blackened, so that the finer details of the intracellular neurofibrils are clearly and sharply outlined.

4. Bielschowsky-Plien Cresyl Violet Method for Nissl Bodies[42]: Splendid stained images of these bodies are secured with this method after fixation in solution of formaldehyde or in alcohol. The bodies assume a brighter color than in paraffin sections, and the duration of staining in cresyl echt violet R is comparatively lessened. There is no visible difference in staining after fixation in solution of formaldehyde or in alcohol, and since the latter renders tissue very brittle by its excessive shrinking action, solution of formaldehyde is recommended as the fixative of choice. The method of Roussy and Lhermitte with 1 per cent toluidine blue and Gothard's fluid as the differentiator may also be employed to advantage.

Histochemical Methods.—The usual methods for the identification of glycogen, chromatin, mucin, epinephrine, calcium salts, iron pigments, etc., can all be successfully applied to sections of tissues impregnated with soap-wax.

Wilder Technic for Reticulum.—The only other method to which reference will be made at this time is the Wilder[43] technic of silver impregnation of reticulum. In soap-wax sections the reticulum fibers tend to present a moderately blacker color than in corresponding paraffin sections, but there is essentially no difference in the time of impregnation (fig. 3).

SUMMARY

The soap-wax technic is superior to the usual paraffin and pyroxylin (celloidin) methods in point of simplicity and time, simultaneously dehydrating and infiltrating tissues in vacuo within two hours after fixation.

By virtue of the relative softness of the soap-wax medium, the absence of crystallinity in it and the reduction in shrinkage and hardening of the tissue, this medium permits sections from 2 to 6 microns thick to be cut without difficulty.

A technic for securing both flattening of sections and their adhesion to glass slides without manipulation is offered.

The presence of a dye solvent, diethylene glycol, accelerates materially the time of staining with synthetic aniline dyes, yielding superior definition of color in stained histologic sections.

42. Bielschowsky, M., and Plien: Neurol. Centralbl. **19**:1141, 1900.
43. Wilder, H. C.: Am. J. Path. **11**:817, 1935.

The fixation image is preserved with fidelity as shown in iron-alum-hematoxylin sections stained according to the method of Martin Heidenhain.

The soap-wax technic is indicated for the study of fine cell structures in that the tissues are not transferred from one fluid to another, thus setting up diffusion currents which can readily lead to injury, excessive shrinkage and unfaithful reproduction of the cellular details.

On the basis of the evidence collected so far, the method is economical, the cost of it being from 30 to 40 per cent less than that of the regular technic.

EFFECT OF STAPHYLOCOCCUS TOXIN ON THE DOG'S BLOOD PRESSURE

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The most important contributions to the study of the effect of staphylococcus toxin on blood pressure are those of Russ [1] and Kellaway, Burnet and Williams.[2] Russ observed in the cat an immediate drop in blood pressure following intravenous injection of staphylococcus toxin. The fall was followed immediately by recovery. Following this recovery a terminal fall occurred in the blood pressure after a short interval. Heart failure accompanied the changes in the blood pressure. The effect of the toxin on the blood pressure was thought to be the result of an obstruction in the pulmonary circulation. Kellaway and his co-workers confirmed the observations of Russ and showed further that intravenous injection of crude staphylococcus agar toxin has a two-fold effect on blood pressure in the cat and the rabbit. They observed, first, a transient fall, followed immediately by recovery. This fall was due to pharmacologically active constituents in the medium. They then observed a second fall, which was due principally to an obstruction in the pulmonary circulation.

In contrast to the findings of these investigators, no pulmonary obstructions were observed by me in a study of the acute pathologic lesions occurring in the dog and the rabbit following intravenous injection of staphylococcus toxin.[3] In view of this pathologic study, and also since no observations have been made on the changes in the blood pressure of dogs receiving staphylococcus toxin, an experiment was performed to determine the effect of this toxin on the carotid blood pressure in the normal and in the immune dog.

METHODS AND MATERIALS

Staphylococcus Used.—The strain of Staphylococcus aureus used for the production of toxin in this investigation was the same as that used in previous

From the Department of Pathology, Vanderbilt University Medical School.

1. Russ, V. K.: Ztschr. f. exper. Path. u. Therap. **18**:220-250, 1916.

2. Kellaway, C. H.; Burnet, F. M., and Williams, F. E.: J. Path. & Bact. **33**:889-912, 1930.

3. Rigdon, R. H.: Arch. Path. **20**:201-208, 1935.

studies.[4] The organism was obtained from the pharynx of a patient with the clinical and pathologic lesions associated with agranulocytic angina.

Preparation of Toxin.—The toxin was obtained from a forty-eight hour growth of this staphylococcus in broth. The broth consisted of veal infusion, 2 per cent proteose peptone, 0.5 per cent sodium chloride, 0.7 per cent disodium hydrogen phosphate (Na_2HPO_4) and 0.3 per cent agar. This broth in 500 cc. amounts was put into flasks holding 2 liters each. The medium was autoclaved. Each flask was then inoculated with 1 cc. of an eighteen to twenty-four hour infusion broth culture. Through a rubber stopper containing two small glass tubes, 20 per cent carbon dioxide in 80 per cent oxygen was slowly introduced into the flasks for from one to three minutes. The flasks were then sealed by clamped rubber tubing and incubated at 37.5 C. for forty-eight hours. After centrifugation the supernatant fluid was filtered through Berkefeld N candles, tested for sterility and stored at 15 C. The p_H of the toxin was 7.6.

Preparation of Controls.—Uninoculated broth and the different constituents in the medium were prepared as separate solutions and used as controls.

The veal infusion was prepared by mixing 500 Gm. of finely minced veal with 1,000 cc. of distilled water. This mixture was left in the chill room for twenty-four hours. The fluid from the meat was pressed through scrim, heated at 50 C. for thirty minutes and then boiled for twenty minutes. The infusion was next filtered through cotton. The p_H was then adjusted to 7.6.

Sodium chloride was prepared in distilled water, in 0.85 per cent concentration.

The proteose peptone was prepared in distilled water, in 2 per cent concentration. The p_H was adjusted to 7.4.

Agar was prepared in physiologic solution of sodium chloride, in 0.3 per cent concentration.

The disodium hydrogen phosphate (Na_2HPO_4) was prepared in physiologic solution of sodium chloride, in 0.7 per cent concentration. The p_H was adjusted to 7.6.

The preparations described were autoclaved and stored at 15 C.

Experimental Technic.—The animals used were ten adult dogs weighing from 8.25 to 16 Kg. Two of these dogs were immunized to staphylococcus toxin by being given several injections of the toxin at different intervals of time. The dogs were anesthetized with either sodium amytal or pentobarbital sodium, administered by either the intravenous or the intraperitoneal route. The carotid artery was cannulated and connected with a mercury manometer. The blood pressure was recorded on a kymograph.

All the fluids were warmed to approximately body temperature before they were injected into the femoral vein. The volume of toxin and of each of the control solutions was always the same for each animal unless otherwise stated. The volume per kilogram of toxin and of control solutions, however, varied for the different dogs.

Several injections of the toxin and of the control solutions were usually given each animal. The experiments were frequently discontinued when the blood pressure reached a level at which the dogs failed to respond to any solution injected.

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4. Rigdon, R. H.; Joyner, A. L., and Rickets, E. T.: Am. J. Path. **10**:425-433, 1934. Rigdon, R. H., and Leff, W. A.: Arch. Path. **21**:298-307, 1936. Rigdon, R. H.: Inhibitory Influence of Hypertonic Solutions of Sodium Chloride on Staphylococcus Hematoxin, J. Infect. Dis., to be published.

EXPERIMENTS

Experiment 1. Effect of the Toxin on Blood Pressure in Normal Dogs.—Four dogs were given a total of five injections of the toxin. The blood pressure fell during or immediately after each injection and returned to approximately the original level after a short interval. The variation in the response of the different dogs to staphylococcus toxin is illustrated in this experiment:

The normal blood pressure in dog 10 was 160 mm. of mercury. He was given six injections of the control solutions before receiving the toxin. The pressure was 90 mm. immediately before 5 cc. of the toxin was injected. Within one minute after this injection was completed the pressure fell to 80 mm., and it returned immediately to 90 mm. In dog 12 the changes in the blood pressure were greater. The normal pressure was 120 mm. of mercury. It was 80 mm. thirty seconds after injection of 9 cc. of the toxin. One minute later it was 180 mm. An increase in the rate and depth of the respirations accompanied these changes in the blood pressure. Dog 13 illustrates another type of change in the blood pressure. The pressure was 120 mm. when the toxin was injected, 80 mm. thirty seconds after completing the injection and 100 mm. sixty seconds later. The curve illustrating these changes is shown in the figure (*B*). In dog 19 the normal pressure was 120 mm. Immediately after completing the injection of toxin the pressure dropped to 60 mm., and during the following sixty seconds it returned to 120 mm. The pressure maintained this level for ninety seconds and then gradually fell to 58 mm. over a period of eight minutes. Blood was found oozing from the wound in the neck at this time. During the next forty minutes the blood pressure gradually increased to 84 mm. The pressure was 80 mm. when the second dose of toxin, approximately twice the volume of the former, was injected intravenously. Sixty seconds after this injection the pressure was 50 mm. Sixty seconds later it was 140 mm. This level was maintained for sixty seconds, and then the pressure began to drop. After six minutes the pressure was 60 mm. The dog was obviously dying. He was dead twenty minutes after the last injection of toxin.

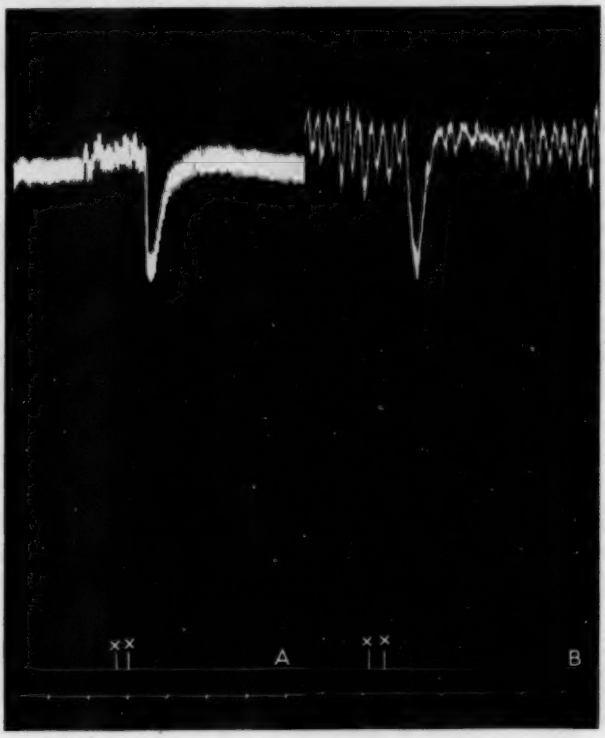
Experiment 2. Effect of the Uninoculated Broth on Blood Pressure in Normal Dogs.—Six dogs were given a total of ten injections of the control solution. The blood pressure fell after seven of these injections and remained unchanged after two. The characteristic change produced in the blood pressure by intravenous injections of the control solution is shown in the figure (*A*). The blood pressure changes in three of the dogs in this experiment were especially interesting.

Dogs 10 and 11 were the two animals in which the pressure remained constant after injection of the control solution. The blood pressure in the former at the beginning of the experiment was 160 mm. of mercury. He received several injections of the solution of proteose peptone before the control solution was given. The pressure was 50 mm. when the last injection of the solution of proteose peptone was made. No change occurred in the blood pressure with this injection. Following this injection 10 cc. of the control solution was given. The pressure did not fluctuate but gradually rose to 60 mm., at which time

20 cc. of the control solution was injected. The pressure fell 10 to 12 mm. during the injection and returned to 85 mm. in one minute after it was completed.

Dog 11 was given three injections of the control solution. A change in the pressure similar to that shown in the figure (*A*) occurred after the first two injections. When the third injection was made the pressure was low, and it maintained a constant level.

The changes which occurred in dog 13 following injection of the control solution duplicated those produced by the toxin. The pressure in this animal was 100 mm. when 10 cc. of the control solution was given over a period of thirty



A, the record of the carotid blood pressure of dog 15 after he had received intravenously 6 cc. of the control solution. *B*, the record of the carotid blood pressure of dog 13 after he had received intravenously 10 cc. of staphylococcus toxin. The space between the x's represents the time of injection. Anesthesia was obtained with pentobarbital sodium.

seconds. The blood pressure immediately dropped to 40 mm. and rose to 100 mm. during the following two minutes. The pressure then gradually fell to 70 mm. over a period of thirty minutes.

Experiment 3. Effect of 2 Per Cent Proteose Peptone Solution on Blood Pressure in Normal Dogs.—Seven dogs were given a total of

sixteen injections of the 2 per cent proteose peptone solution. The blood pressure fell immediately after eleven of these injections and maintained a constant level after five. The characteristic changes in the blood pressure following these injections were similar to those shown in the figure. Three of the five times that no change in the blood pressure occurred the same dog was involved.

This animal was used in determining the minimum volume of the 2 per cent proteose peptone solution necessary to produce a change in the dog's blood pressure. Volumes of 1 and 2 cc. failed to produce any change. When 5 cc. was given the blood pressure dropped 100 mm. in ten seconds. In the fourth injection 15 cc. of the proteose peptone solution was given. With this quantity no change occurred. The pressure at this time, however, was much lower than it was during the preceding injections. The blood pressure in the other dogs was likewise low when no change occurred following injections of the proteose peptone solution. Similar changes were obtained with the toxin and the control solutions when the pressure was low.

Experiment 4. Effect of 0.3 Per Cent Agar Solution on Blood Pressure in Normal Dogs.—Two dogs were given four injections of the agar solution. No change occurred in the blood pressure after any of these injections.

Experiment 5. Effect of Veal Infusion on Blood Pressure in Normal Dogs.—Four dogs were given five injections of veal infusion. The blood pressure dropped after two of these injections and remained constant after three. A change in the pressure was observed in two of the dogs. The curves produced were similar to those shown in the figure. The two animals in which no change in blood pressure occurred after injections of veal infusion did, however, show fluctuation in the blood pressure when other solutions were injected subsequent to the injections of the veal infusion.

Experiment 6. Effect of 0.7 Per Cent Disodium Hydrogen Phosphate (Na_2HPO_4) on Blood Pressure in a Normal Dog.—One dog was given two injections of 0.7 per cent disodium hydrogen phosphate (Na_2HPO_4). The blood pressure did not vary after either injection.

Experiment 7. Effect of 0.85 Per Cent Solution of Sodium Chloride on Blood Pressure in Normal Dogs.—Two dogs were given three injections of physiologic solution of sodium chloride. There was no change in blood pressure after any of the injections. In one dog the volume of saline solution injected was greater than the amount of toxin which produced a change in the pressure.

Experiment 8. Effect of the Toxin and of Control Solutions on Blood Pressure in Immune Dogs.—Two immune dogs were given seven injections of the toxin. The blood pressure fell following six of these injections. The type of curve produced was a duplicate of the type

previously described for the normal dog. One of the two animals was given a single injection of the control solution. The change which occurred in the pressure was essentially the same as that observed in experiment 2 and shown in the figure (*A*). The blood pressure also fell following injection of the proteose peptone solution. There was no change in the pressure following any of three injections of the veal infusion. Likewise no change occurred in the pressure following an injection of the solution of disodium hydrogen phosphate or one of the agar solution.

COMMENT

This paper shows that the blood pressure of normal and immune dogs falls during or immediately after intravenous injection of staphylococcus toxin. Furthermore, a similar change occurs in the blood pressure of normal and immune animals following intravenous injection of preparations of the constituents of the culture medium, as controls.

Proteose peptone and veal infusion, both constituents of the medium in which the toxin is made, produce a change in the blood pressure of normal and immune dogs similar to that produced by staphylococcus toxin.

The response to the different preparations varies in the same dog and also in different dogs. This variation is usually observed when there is a difference in the level of the blood pressure at the time that the test solution is injected. Sometimes when the pressure is very low no change occurs with toxin, control solution or proteose peptone. Frequently a dog shows a drop in blood pressure in response to the second injection of a solution after failing to show any change with the first. This is usually true when the pressure is higher at the time of the second injection than it was at the time of the first. This variation in blood pressure in different dogs following injections of the different preparations was shown by dogs 10 and 11 in experiment 2. The observation made in this study that the blood pressure varies in different dogs coincides with the statement of Watanabe. He stated that "not only different species of animals, but perhaps also different individuals of the same species vary in their susceptibility to peptone administration." [5]

The type of change in the blood pressure often varies in the same animal with either the toxin or the control solution. When first the preparation is injected the pressure falls immediately and requires several minutes to return to the normal level. When the same preparation is injected subsequently the pressure falls immediately and within approximately one minute returns to the normal level. This type of curve is represented in the figure (*A*).

5. Watanabe, M.: Tohoku J. Exper. Med. **9**:412-453, 1927.

The observations made in this study on the changes in the blood pressure of the normal dog following injections of staphylococcus toxin, control solution, proteose peptone and veal infusion are especially interesting when considered along with the observations of Dale and Richards [6] on the vasodilator action of histamine. The effects of histamine and of the preparations used in this study on the blood pressure of the normal dog are similar.

The observation that the same changes occur in the blood pressure of immune and normal dogs following injections of toxin suggests that the changes are not the result of the action of the toxin per se. It appears at this time that until staphylococcus toxin can be obtained free from tissue fluids and peptone it will be very difficult and even impossible to determine whether or not the changes in the circulation are due to staphylococcus toxin or to some constituent in the medium. The evidence suggests that the changes in the blood pressure are due to something other than the toxin.

The results obtained in this work on the effect of staphylococcus toxin on the blood pressure of the normal dog do not agree with the findings of Dicker.[7] He failed to note any change in the pressure of a single dog given daily injections of staphylococcus toxin for fifteen days. The toxin was prepared in ordinary bouillon medium. His technic, however, differed radically from that used in the present investigation.

SUMMARY

Staphylococcus toxin, the control solution and certain constituents in the medium in which staphylococcus toxin is prepared produce similar effects on blood pressure in the normal and in the immune dog. It is suggested that the action of the preparations mentioned is like that of histamine.

Since the control solution produces the same effect on blood pressure as staphylococcus toxin it is apparently impossible to determine whether or not the changes in the circulation following intravenous injection of staphylococcus toxin are the result of the action of the toxin or of that of some constituent in the medium. The evidence suggests that the changes in blood pressure are due to something other than toxin.

6. Dale, H. H., and Richards, A. N.: J. Physiol. **52**:111-165, 1918.
7. Dicker, E.: Compt. rend. Soc. de biol. **120**:793-795, 1935.

PHOTODYNAMIC EFFECTS OF FLUORESCENT SUBSTANCES IN THE LIVING KIDNEY

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Very little is known about the way substances with photodynamic properties exert their action on the living tissue. Nearly all substances with the property of fluorescence have, however, some photodynamic action, and among them are a number of important therapeutic and diagnostic agents. Photodynamic action is responsible for most of the symptoms in some skin and nutritional diseases—for instance, pellagra and xeroderma pigmentosum. It is therefore of interest that observations contributing to the explanation of this phenomenon should be reported.

Records in the literature show that various experiments with photodynamic substances have been made. Tappeiner [1] observed that Paramecia are killed and soon disintegrate after becoming photosensitized. Higher animals possess more resistance, but they also succumb if they are sensitized and exposed sufficiently long to sun or ultraviolet radiation. Pfeiffer [2] found in these animals a high grade hyperemia of the viscera, hemorrhagic gastro-enteritis and fatty degeneration of the kidneys and liver. The gallbladder is filled with bile to the point of rupture, and gangrene of the ear develops if the animals live longer. According to Kögel,[3] photodynamic effects manifest themselves in excitement and muscle contraction in the beginning, followed by paralysis.

This report on photodynamic effects is concerned with observations on changes within the living cells. For some years past I have conducted experiments on fluorescence microscopy of living organs with a method that permits the observation of changes in the living cells and, though it allows visualization, does not interfere with their structure or function.[4] The arrangement allows one to see cytologic details with great

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Aided by a grant from the Diamond Jubilee Fund.

From the Biological Laboratory, Cold Spring Harbor, L. I., and St. Clare's Hospital, New York City.

1. Tappeiner, H., in Abderhalden, E.: Handbuch der biologischen Arbeitsmethoden, Berlin, Urban & Schwarzenberg, 1923, sect. 4, pt. 7, no. 1, p. 1070.

2. Pfeiffer, H., in Abderhalden, E.: Handbuch der biologischen Arbeitsmethoden, Berlin, Urban & Schwarzenberg, 1923, sect. 4, pt. 7, no. 1, p. 1102.

3. Kögel, G., in Abderhalden, E.: Handbuch der biologischen Arbeitsmethoden, Berlin, Urban & Schwarzenberg, 1931, sect. 2, pt. 2, no. 2, p. 1999.

4. Singer, E.: Science **75**:289, 1932; Anat. Rec. **58**:93, 1933.

clearness, as the numerical apperture of the objective is 0.80, and the total magnification, × 600. A great number of dyes and other substances, such as alkaloids and glucosides, have been examined. Most of them show very slight and gradual photodynamic effects. Two of the substances, however, thioflavine S and berberine sulfate, showed sudden and striking effects, yielding some information on the manner in which the photodynamic action occurs.

Thioflavine S is a yellow aniline dye that is readily soluble in water and alcohol, yielding a yellow color, and in sulfuric acid, in which it gives a brownish yellow color. In ultraviolet rays the fluorescence of

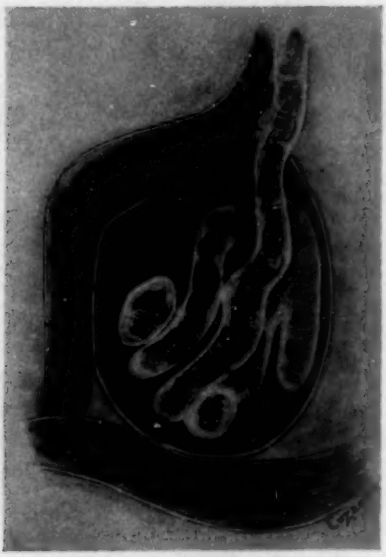

Fig. 1.—Malpighian body of a living frog in fluorescence staining by thioflavine S after exposure to ultraviolet radiation for more than five minutes, showing the faded fluorescence of thioflavine S and stasis in the glomerular capillaries (from Singer, E.: *Anat. Rec.* **66**:1, 1936).

this dye in neutral solutions and alkaline solutions is blue. The solid form shows orange fluorescence in ultraviolet rays, and acid solutions show no fluorescence. If 1 cc. of a 4:1,000 solution is injected intramuscularly for each 100 Gm. of the weight of a frog, it shows the cell nuclei in a bright blue fluorescent light. In the blood stream the fluorescence of thioflavine S is yellow. In the capillary wall of the glomerulus the substance appears in a greenish blue fluorescence. After ultraviolet irradiation of the glomerulus for from three to five minutes, invariably a typical change occurs; first, the brilliancy of the color disappears, and the loops become dull; then the greenish blue capillary

wall turns to orange-yellow, and at the same time, when this change of color takes place, the efferent vessel of the glomerulus contracts, and because the afferent vessel does not change its caliber the capillaries become filled with blood corpuscles that stream in through the afferent vessel. The color change affects all vessels that are exposed to ultraviolet radiation, leaving unaffected the nonirradiated ones. Even more prolonged irradiation does not seem to cause color changes in other cells of the kidney. This observation shows that some interference occurs within the cells of the vascular system, the interference manifesting itself by causing a spasm in a definite place, notably in the

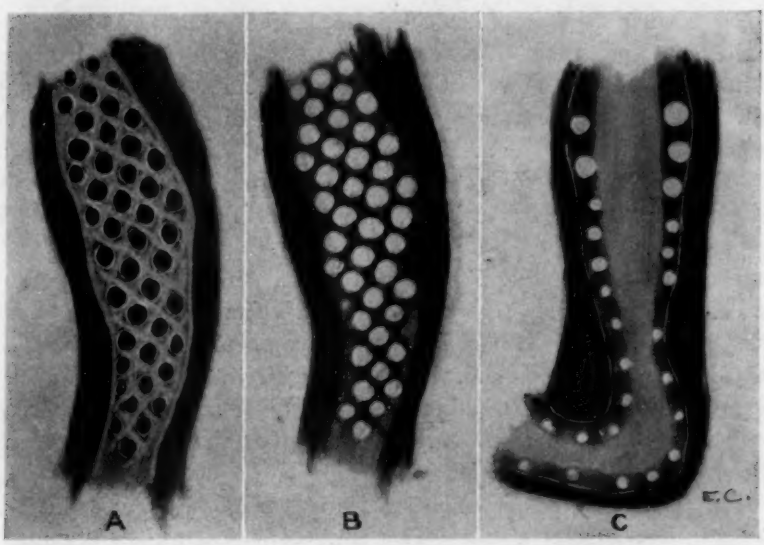

Fig. 2.—*A*, tubule in the living kidney of a frog as it appears in the fluorescence of berberine sulfate. The regular distribution of the nuclei is striking. They appear dark surrounded by bright cytoplasms. As fluorescence staining is emission of fluorescent light from the stained part, it is evident in this case that the cytoplasm is stained. *B*, the same tubule after the tissues had been irradiated by ultraviolet rays for five minutes. The staining is reversed. *C*, tubule in the living kidney of a frog in fluorescence staining by thioflavine S. Note the similarity of this picture to *B*.

efferent vessel, and that the blood serum, which inhibits the effects of photodynamic action in vitro, does not have the same inhibiting property in vivo. It seems, rather, that blood serum is more sensitive to the effect of thioflavine S than cells in the organs (figs. 1 and 2 *C*).

Berberine sulfate is a yellowish acridine dye that can be extracted from the bark of Hydrastis canadensis. In Europe it is found in the bark of Berberis vulgaris. It is the only natural dye that is alkaline.

Its fluorescence is yellow in neutral and green in alkaline solutions; when it is in solid form its fluorescence is yellow, and it has no fluorescence in acid solutions. If 1 cc. of a 1:1,000 solution is injected intramuscularly for each 100 Gm. of the weight of a frog the endothelial cells of the blood vessels appear with a yellowish fluorescence. The cytoplasm of other cells has the same color, and the nuclei appear as round dark spots. During irradiation this picture goes through changes. Within from two to three minutes the picture gradually becomes hazy; it is impossible to see the boundaries of the nuclei or the boundaries of the cytoplasm of the individual cells, as the staining is diffused uniformly over the entire kidney. If the irradiation is prolonged further the cytoplasm of the cells fades out and the nucleus assumes the appearance of a very brilliant yellow fluorescence. Careful examination reveals now that the cell membranes are colored in the same way, forming extremely thin boundary lines between the cytoplasms of the cells. Very thin fibers of the same color are visible within the cytoplasm, but as yet I am unable to determine their character. Nonirradiated parts of the same kidney remain unaffected (fig. 2 *A* and *B*).

The phenomenon described shows that a definite change occurs in the cells of the kidney sensitized with berberine sulfate. This change takes place after the kidney has been exposed to ultraviolet rays and manifests itself in a reversal of the staining from cytoplasm to nucleus. I cannot say at present whether this reversal of staining by disturbing the metabolism of the cell changes the p_H or the electropotential of its elements, as both could be the case.

The photodynamic phenomena observed with these two dyes show results of direct effects of ultraviolet rays on photosensitized organs.

SIZE AND WEIGHT OF THE NORMAL AND OF THE PATHOLOGIC PROSTATE GLAND

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The subjects of the etiology and the treatment of prostatic hypertrophy have been given generous space in the literature since the original description of the condition by Morgagni.[1] However, very little has been said about the distinction between the normal and the pathologic prostate gland from an anatomicopathologic point of view. Nevertheless, distinguishing between them from this point of view is essential in statistical studies for determining the incidence of prostatic hypertrophy in the general male population, particularly as exemplified at postmortem examination.

OBJECT OF STUDY

In a recent study of prostatic hypertrophy[2] it was found necessary to classify a large number of glands as normal or pathologic. At the same time the opportunity was offered to measure and weigh these glands and to record the results.

The chief purpose of the part of the study which is reported here was to determine the weights and the measurements of the glands which, by gross inspection only, had been classified as normal, atrophic or hypertrophic; the last group was further subdivided into four grades. It was hoped that the glands of each group might fall within a definite range of weight so that definite qualifications on the basis of weight might be set for inclusion of prostate glands in the various groups. Moreover, the measurements were compared with the weights to see if any relationship existed between the two. It was hoped that some method might be devised for calculation of the weight from the measurements.

This work was done under the direction of Dr. H. E. Robertson.

1. Morgagni, J. B.: The Seats and Causes of Diseases Investigated by Anatomy, translated from the Latin by Benjamin Alexander, London, A. Millar & T. Cadell, 1769, vol. 3, pp. 460-462.

2. Teem, M. V.: J. Urol. **34**:692-713, 1935.

MATERIAL AND METHOD

The material used in this study was taken at consecutive postmortem examinations of the bodies of one hundred men. By avoiding selection of material, any tendency to overburden the group with hypertrophied prostate glands was obviated. The prostate glands had not been a factor in the deaths of any of the patients. The subjects ranged from 20 to 89 years of age at death, and there was a fairly even distribution of these subjects among the various decades of life. The number of patients in each decade, from the second to the eighth, was five, twelve, fifteen, twenty-nine, twenty-six, nine and four, respectively. There was a slight preponderance of patients who were between the ages 40 and 70 years at death; the average age for the group as a whole was 54.3 years.

The prostate gland was carefully dissected and accurately weighed at the time of the postmortem examination. As has been said, from gross appearance only, the glands were classified in the various groups. Having thus been classified, the extreme weights and measurements in each group were determined.

Comparison of the weights with the measurements in each group led to the belief that some correlation existed between the measurements and the weights. Because the prostate gland fails to conform with any geometric figure, however, it was realized that an attempt to calculate the weight from the measurements could be made only on the basis of values empirically determined. It was decided to determine a factor, x, which when multiplied by the product of the multiplication of the three dimensions of the prostate gland would equal the weight. Each gland was considered separately in these calculations, and by a process of trial and error a certain value for the factor x was found. In a large majority of the cases, use of this factor resulted in a calculated weight agreeing with the actual weight within satisfactory limits of error.

NORMAL PROSTATE GLAND

Thirty-three of the one hundred specimens were classified as normal. The weights for this group, as shown in the table, compare favorably with the values of from 20 to 25 Gm. given by McMurrich.[3]

Piersol [4] stated that the average weight of the normal prostate gland is 22 Gm., while Wallace [5] placed the average weight at 20.5 Gm. As closely as these figures seem to agree, it should be remembered that a difference of from 2 to 3 Gm. is readily discernible to the trained eye. An idea of the variation in weight and of the average weight of normal glands as compared with atrophic and with hypertrophic glands is readily obtained from figure 1.

The dimensions recognized are length, the greatest dimension in the direction of the axis of the urethra; width, the greatest dimension from side to side, and depth, the greatest dimension in the anteroposterior direction. The dimensions recorded in figure 2 agree with the general observation that the greatest dimension is the width; the length is next,

3. McMurrich, J. P., in Morris, H.: Human Anatomy, ed. 5, Philadelphia, P. Blakiston's Son & Co., 1914, p. 1265.

4. Piersol, G. A.: Human Anatomy, ed. 7, Philadelphia, J. B. Lippincott Company, 1919, pp. 1975-1977.

5. Wallace, C. S., quoted by Lowsley, O. S.: Ann. Surg. **62**:716-737, 1915.

and the smallest dimension is the depth. However, of normal glands as well as of the glands in the other groups, to be discussed later, the depth is relatively greater than that given by most observers, who report a depth of less than 2 cm. Wallace gave the average length, width and depth as 3, 3.6 and 1.8 cm., respectively, and the average weight as 20.5 Gm. The last is a slightly lower figure than that given either by McMurrich or by Piersol. Thompson,[6] on the other hand, gave about the same average dimensions as Piersol and McMurrich gave, namely, 3.5, 4.3 and 1.75 cm., respectively. Thompson,[6] however, gave the average weight as 18.5 Gm., a lower value than that given by Piersol and by McMurrich.

Measurements of Prostate Glands Examined

		Length, Cm.		Width, Cm.		Depth, Cm.		Actual Weight, Gm.		Calculated Weight, Gm.
Type of Gland	Cases	Extremes	Average	Extremes	Average	Extremes	Average	Extremes	Average	Average
Atrophic.....	12	2 to 4.2	3.18	1.8 to 4.1	3.3	1.7 to 3.5	2.35	10 to 17	14.6	16.4
Normal.....	33	2.1 to 3.7	3.08	1.7 to 4.7	3.84	1.1 to 6	2.7	18 to 23.5	20.7	21.2
Hypertrophic, grade 1	31	1.5 to 4.5	3.51	2.5 to 5	4.24	1.9 to 6	3.0	24 to 34.5	29.1	29.76
Hypertrophic, grade 2	15	2.8 to 5.2	3.99	3.6 to 6	4.78	2.3 to 4.3	3.28	35 to 51	40.6	41.7
Hypertrophic, grade 3	6	4.2 to 5.9	4.87	4.5 to 6.5	5.33	2.5 to 4.5	3.63	54 to 71	61.3	62.78
Hypertrophic, grade 4	3	4.5 to 6	5.26	6 to 6.7	6.4	4.9 to 5.7	5.2	112 to 139	129.6	116.68
Total hypertrophic	55	1.5 to 5.9	3.89	2.5 to 6.7	4.62	1.9 to 5.7	3.27	24 to 139	41.2	39.17
All types.....	100	1.5 to 5.9	3.54	1.7 to 6.7	4.2	1.1 to 6	2.96	10 to 139	31.24	29.32

Although there was a relatively larger number of normal prostate glands among the patients who died at ages younger than 40 years as compared with patients who died later in life, there were several patients of each decade of life.

ATROPHIC PROSTATE GLAND

There is a very gradual transition between the normal and the pathologic prostate gland, even when viewed at necropsy. I believe, however, that atrophic glands are probably more easily distinguished from normal ones than are glands which have undergone the finer grades of enlargement. In fact, atrophic glands appear much more reduced in size than the measurements indicate. The average measure-

6. Thompson, Henry, quoted by Lowsley, O. S.: Ann. Surg. **62**:716-737, 1915.

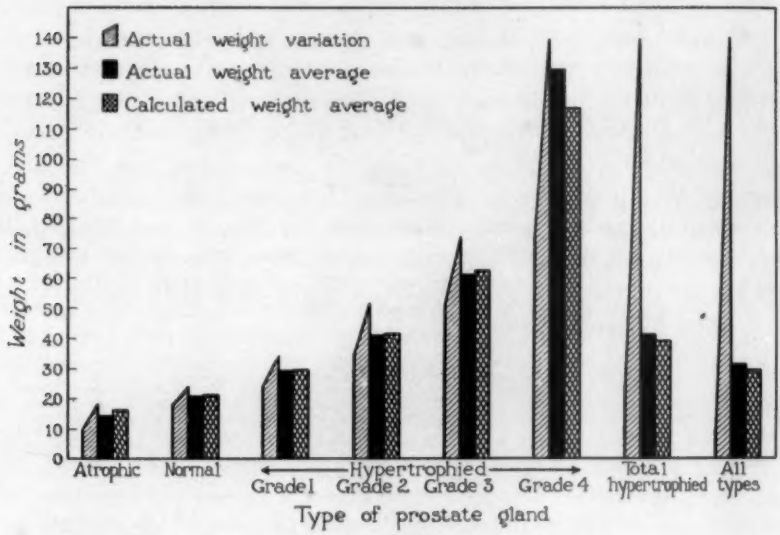

Fig. 1.—Variation in actual weight, average actual weight and average calculated weight of the various types of prostate gland (atrophic, normal and hypertrophied).

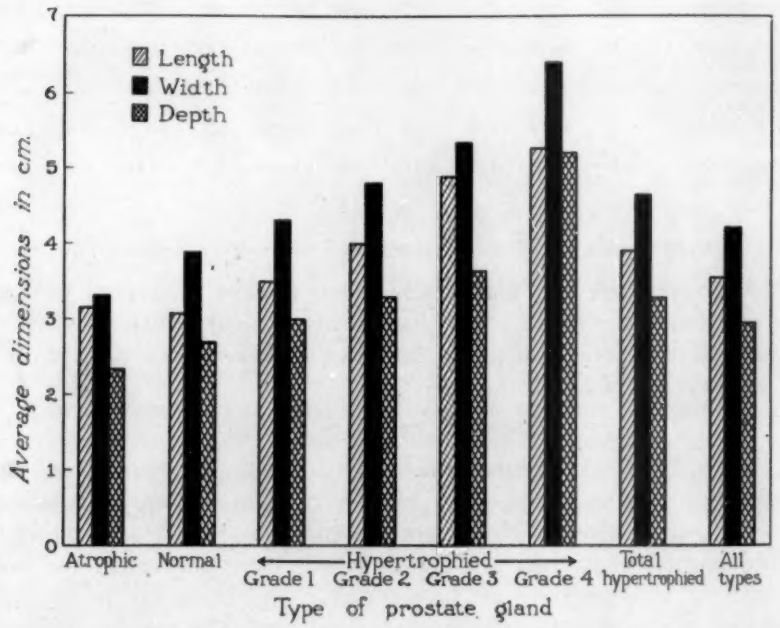

Fig. 2.—Average dimensions of the various types of prostate gland (atrophic, normal and hypertrophied).

ments of the atrophic glands concerned here were only slightly less than those of the normal glands, as is evident in the table. In general, the atrophic prostate gland seems to be smaller mainly in the antero-posterior dimension (depth), whereas the length is not reduced. In fact, in the present series the length of the atrophic glands as a whole was slightly greater than that of the normal glands.

A gland which weighs 10 Gm. is small indeed; this is the weight of the gland usually seen at about the age of 12 years. When such a gland is found in an adult, it is usually associated with atrophic testes, such as are seen when there is pituitary hypofunction or after mumps or traumatic orchitis.

HYPERTROPHIED PROSTATE GLAND

Fifty-five of the one hundred specimens appeared larger than normal. While the weights of these glands varied between 24 and 139 Gm., it is well known that much larger prostate glands are found. Alcock [7] reported that some prostate glands of man, removed surgically, weighed as much as 290 Gm. Incidentally, the average weight of 433 prostate glands removed surgically by Alcock was 38.3 Gm.; this is striking in view of the fact that the average weight of the fifty-five hypertrophied glands concerned in the present report was 41.2 Gm. Prostate glands weighing from 200 to 300 Gm. not uncommonly have been removed by prostatectomy at the Mayo Clinic (Thompson [8]).

The size of the prostate gland may have little to do with the presence of clinical symptoms or with their degree. The subject in this series whose prostate gland weighed most, namely, 139 Gm., gave no evidence of urethral obstruction at the time of postmortem examination and no history of significant genito-urinary symptoms. The weight of the glands representing each of the four grades of prostatic hypertrophy is represented in figure 1. The weights representing hypertrophied prostate glands of the four grades were as follows: grade 1, from 24 to 34 Gm.; grade 2, from 35 to 51 Gm.; grade 3, from 52 to 74 Gm., and grade 4, 75 Gm. and more. Considering all the enlarged prostate glands as a group, the average dimensions of 3.9, 4.6 and 3.2 cm. for length, width and depth, respectively, do not seem much greater than those for the normal prostate glands. This is only one of the several reasons why weight is much more desirable than measurement for classifying a prostate gland. A more valuable piece of information, however, as far as the measurements are concerned, is that variation occurs in each dimension for each grade of enlargement, as is shown in figure 2.

The most unusual finding in regard to the hypertrophied glands was their relatively great depths, as much as 5.2 cm. This bears out

7. Alcock, N. G.: J. A. M. A. **104**:734-738, 1935.
8. Thompson, G. J.: Personal communication to the author.

the observation made at the time of the examinations that the prostate gland tends to round out as enlargement takes place, changing from the more or less flat, chestnut-like shape to a more spherical shape.

CALCULATION OF THE WEIGHT OF THE PROSTATE GLAND FROM THE MEASUREMENTS

The most accurate and satisfactory method of gaging the size of an organ is by weight. This method is generally used in determining the size of the heart, the liver and the brain. Weighing of the prostate gland, however, entails its careful dissection from the bladder. To avoid this cumbersome and time-consuming procedure, a method was sought to calculate the weight of the gland from its measurements.

By a process of trial and error it was found that the product of the multiplication of the three dimensions multiplied by the factor 0.666 gave a value which represented the actual weight with considerable accuracy. In the table the actual and the calculated weights of the glands are compared, while in figure 1 the results are represented graphically.

SUMMARY AND CONCLUSIONS

From subjects ranging in age from the third to the ninth decade of life one hundred prostate glands were carefully dissected, measured and weighed.

These glands were classified either as normal or as having undergone pathologic change, on the basis of general appearance, and the pathologic ones were then classified according to whether they were atrophic or hypertrophic. The hypertrophied glands were graded 1 to 4 on the basis of size; grade 4 represented the largest and grade 1 the smallest glands.

The average dimensions of the prostate glands of the various groups given in centimeters in the order of length, width and depth were as follows: atrophic glands, 3.18, 3.3 and 2.35; normal glands, 3.08, 3.84 and 2.7; hypertrophic glands, all grades taken together, 3.89, 4.62 and 3.27.

The weights for the glands of each group were determined, and since they fell quite definitely into certain ranges it is proposed that, from the anatomic point of view at least, the prostate gland of the adult be considered as follows: normal if it weighs from 18 to 23 Gm. inclusive; atrophic if it weighs 17 Gm. or less, and hypertrophied if it weighs 24 Gm. or more.

A fairly constant relation between the dimensions and the weights for each group of prostate glands was discovered.

The weight of the prostate gland can be calculated within a reasonable degree of accuracy from the dimensions.

Case Reports

PRIMARY LIPOSARCOMA OF THE GREAT OMENTUM

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While a mesoblastic malignant tumor associated with the peritoneum, springing from its lining cells, its folds or its recesses, is not entirely uncommon, the origin of such a tumor in the great omentum is relatively rare. In 1934 Ransom and Samson [1] reviewed the literature concerning primary tumors of the great omentum and found reports of seventy-five instances. Of these, thirty-three were listed as sarcoma, thirteen as fibrosarcoma, eight as myosarcoma, eight as angiosarcoma, seven as endothelioma, five as carcinoma (so-called) and one as myofibrosarcoma. The chronological chart presented by these authors discloses a predominance in the third, fourth and fifth decades of life, 50 per cent occurring before the age of 40. It is also noteworthy that most of the tumors were composed of spindle cells, though all were definitely of mesoblastic origin with the exception of the instances of so-called carcinoma. The latter may have been instances of mesothelioma. To the aforementioned reports the authors added their own of an instance in a 27 year old Negro woman who, in addition to the other symptoms of pain, fatigue, sense of weight and distention of the abdomen, had marked secondary anemia. Paracentesis gave a bloody fluid with 2,450,000 red cells and 2,700 white cells. The hemoglobin content was 29 per cent, and the icteric index, 100. All of the latter observations were significant since at the operation there was removed a large hemangioma cavernosum with proliferation of the endothelium of the omentum (C. V. Weller, pathologist). The patient was still living at the time of the report.

In addition to these contributions there are reports of primary sarcoma of the omentum by McDonald,[2] lymphangio-endothelioma and reticulo-endothelioma gastromentale by Fogliani,[3] mesothelioma malignum by Masaguma,[4] reticular cell sarcoma by Sanes and Kenny [5] and multiple sarcoma of the omentum by Caligaris.[6] The latter diagnosis seems to us somewhat questionable because of the small nodules near the tail of the pancreas and the limited involvement. There are, therefore, about eighty-three instances of apparent primary tumors of the omentum

From the Pathological Laboratory of the Veterans' Facility and the Department of Pathology of the University of Oregon Medical School.

1. Ransom, H. K., and Samson, P. E.: Ann. Surg. **100**:523, 1934.
2. McDonald, A. L.: Arch. Surg. **14**:1245, 1927.
3. Fogliani, U.: Arch. ital. di anat. e istol. pat. **2**:493, 1931; Pathologica **24**: 463, 1932.
4. Masaguma, M.: Okayama-Igakkai-Zasshi **45**:1838, 1933.
5. Sanes, S., and Kenny, F. E.: Am. J. Cancer **21**:795, 1934.
6. Caligaris, E.: Cancro **5**:150, 1934.

recorded in the literature. Not all of these were definitely proved to be instances of primary tumor of the omentum, nor were they all correctly classified as to cell type. Accordingly, in view of the rarity of the lesion, the present case being the first in our experience, the following report is made:

A mail carrier aged 40 was admitted to the hospital on Feb. 20, 1935. He stated that he had been well until January. At that time he noticed a swelling of the abdomen. After being under observation, he left the hospital and went to the Mayo Clinic. There it was found that he had ascites of a marked degree and

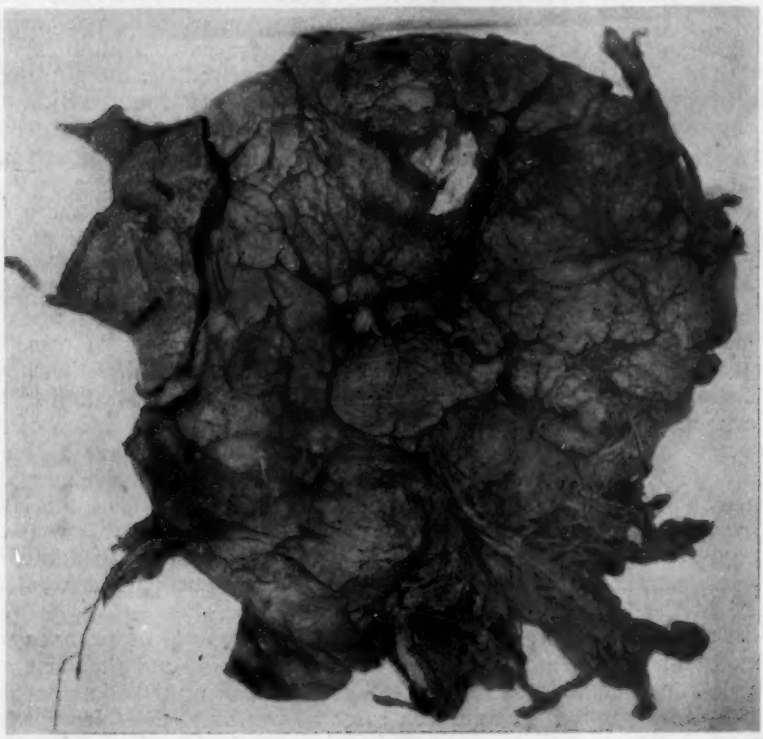

Fig. 1.—The larger portion of the mass in the great omentum. Note the characteristic coarse lobulation, which is not unlike the natural accumulations of fat found in the region of the omentum.

secondary anemia. By paracentesis 8,000 cc. of a very bloody fluid was removed. Following this removal, masses were felt in the upper abdominal quadrants and in the epigastrium. It was also discovered that there was an absence of renal function on the left side and that the right kidney was enlarged. The condition was regarded as probably abdominal carcinomatosis, with the primary growth in the liver, and nothing further was done. At the time of the second admission, June 26, the patient appeared fairly well nourished but looked ill. He weighed 179 pounds (81.2 Kg.), which was about 10 pounds (4.5 Kg.) under his usual weight. His blood pressure was 160 systolic and 80 diastolic. He was examined

by Dr. O. B. Wight in the tumor clinic of the hospital, and the diagnosis concurred with that made previously. On June 29, by paracentesis 3,000 cc. of bloody fluid was removed; six days later, 6,680 cc. and fifteen days after this, 6,000 cc. The urinalysis gave essentially negative results; the blood count showed 4,090,000 red and 9,300 white cells, with a fairly normal differential distribution. The gastric analysis revealed no free hydrochloric acid. The fluid removed from the abdomen was said to be highly colored with bile. The cultures were negative. The patient died Sept. 15, 1935, approximately nine months after the onset of the symptoms.

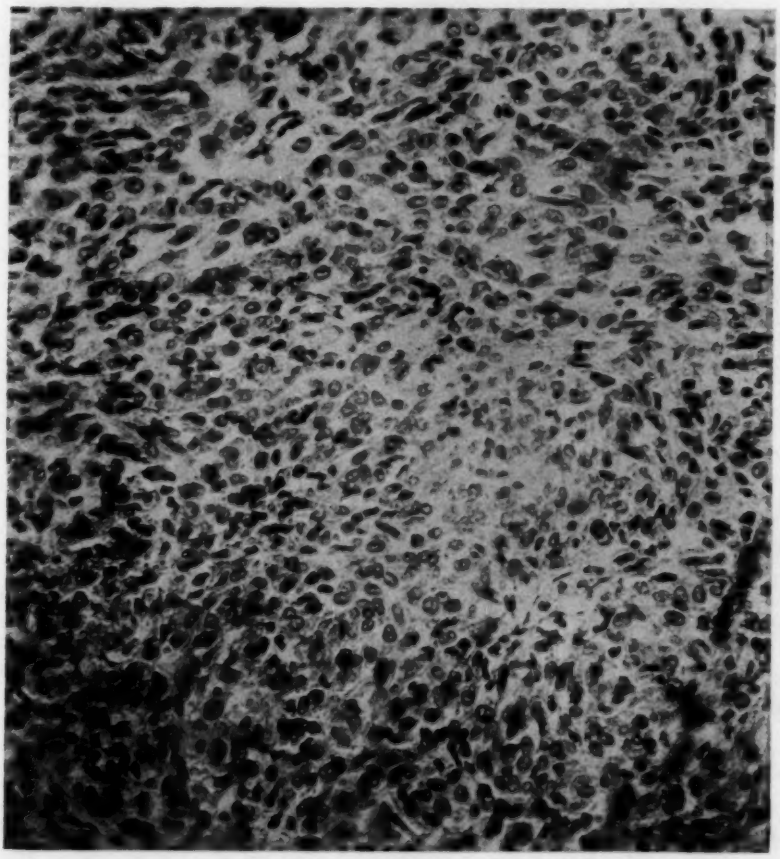

Fig. 2.—One of the more cellular areas in the original tumor. Observe the variation in appearance of the cells and their nuclei. When such sections were stained with sudan III, the presence of fat granules could be noted in many of the cells. These granules were less numerous in the more anaplastic areas.

At autopsy (F. R. M.) the body was generally wasted, with prominent bony landmarks. The abdomen bulged, with the umbilicus everted. In the peritoneal cavity the viscera were seen pushed forward. There was a distinct odor of putrefaction. The lower border of the liver was at the costal border, and there was no marked enlargement. The transverse colon and the stomach were found

above the large mass which filled the abdominal cavity. The loops of the small bowel were not distinguishable as such. In what appeared to be the great omentum there was a diffuse infiltrating new growth, which was nodular and partially necrotic. Flecks of fibrin were scattered in the abdomen, especially about the wounds of the paracenteses, and where hemorrhage had occurred only a moderate amount of bloody fluid was present. A number of coils of the small intestine were crowded upward in the region of the left hypochondrium, in the vicinity of the spleen. Some of these loops were bound together by necrotic tumor masses, which appeared to be secondarily implanted. All of the tumor about

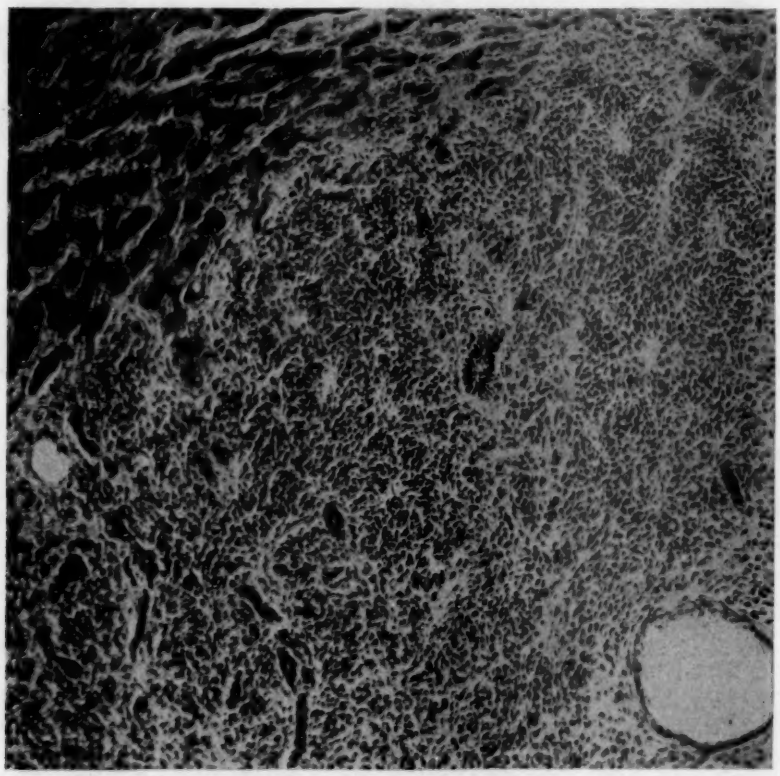

Fig. 3.—Metastasis in the liver. The sarcomatous spindle cell nature is evident even in the low magnification.

the small bowel, as well as that seen about the descending colon, was definitely anterior to the posterior peritoneum. The great omentum was occupied by a diffuse yet nodular new growth that retained the omental pattern (fig. 1). When this mass was sectioned, there were revealed circumscribed nodules with central areas of cystic and hemorrhagic degeneration, and scattered throughout were small semipurulent foci of necrosis. In addition to the large mass in the omentum and that about certain loops of the intestines, a number of nodules were present in the liver. One of these was in the right lobe, near the inferior margin. Its center was degenerated, forming a bloody cystic mass 11 cm. in diameter. Other nodules were present, varying from 1 to 3 cm. in diameter. The remaining liver

tissue was pale brownish. These nodules in the liver were distributed in both lobes. There was no evidence of involvement of any of the other organs, including the stomach, pancreas, large and small bowel and spleen. There was no evidence of extension beyond the diaphragm. The left kidney was congenitally absent, and there was a one-sided development of the trigon of the urinary bladder. The right kidney was enlarged, weighing 853 Gm. It had many cysts throughout, which were characteristic of those in polycystic kidneys.

Microscopic sections disclosed lawlessly arranged tumor cells, which almost completely obliterated all of the fat cells. In most of the sections, the stroma was rather delicate, broadening out about the blood vessels, which were numerous. In all portions of the tumor the cells were in relationship to blood vessels, the larger arteries as well as the small arterioles, sinusoids and veins. In the walls of some of these vessels there were radially arranged tumor cell structures which occupied the entire adventitia, forming variable-sized perivascular collars. No invasion of the intima was observed. The cells around such blood vessels were often arranged in palisades, especially in the vicinity of the veins. In addition, there were relatively large areas composed of cells filled with lipoid material, as was demonstrated by special stains. The cytoplasm of many spindle cells contained lipoid granules. There was less evidence of fat in the more anaplastic areas. In still other parts there were extensive hemorrhages, with blood pigment changes, associated with secondary infection, as indicated by the presence of large numbers of polymorphonuclear leukocytes. The characteristic type cell was predominatingly spindle shaped (fig. 2). The cells in the metastases to the liver were similar (fig. 3). In the original tumor an occasional multinucleated giant cell could also be seen. Only an occasional mitotic figure was found, although hyperchromatism was generally evident. The metastatic nodules in the liver consisted of typical groups of so-called spindle cells lying near the hepatic trinities. The liver cells appeared pushed back and compressed about the periphery. There was also some proliferation of the bile ducts.

COMMENT

The relatively sudden onset of symptoms and the short course, as well as the fatigue, pain and feeling of fulness, together with the rapidly forming bloody ascites and the associated secondary anemia, are the outstanding clinical characteristics of the case reported. It is also significant that the man was only 40 years old. There is a tendency for such tumors to occur in the earlier decades of life. There may be, therefore, some significant interrelation between the presence of the congenitally cystic right kidney, the congenital absence of the other kidney and the liposarcoma in the omentum.

The clinical features of the tumor in our case are similar to those reported in many other cases and summarized by Ransom and Samson.[1] According to these authors, the following is the order of frequency of the symptoms: pain in the abdomen; tumor and sense of weight in the abdomen; ascites; weakness and loss of weight; distention of the abdomen; constipation or diarrhea or both; anemia. Occasionally, there may be symptoms of appendicitis (McDonald [2]). Secondary involvement of the bowel may release micro-organisms from the intestine, as in the instance cited. The frequency of bloody fluid in the abdomen attests to the vascularity of most of the primary tumors of the omentum, regardless of the cell type reported. The occurrence of abnormal blood vessels or the natural vascularity of the omentum probably accounts for

the readiness with which secondary degenerations release blood from the vessels. This hemorrhagic tendency is responsible for the secondary anemia. It is noteworthy that no clinical jaundice occurred in spite of the high icteric index of the ascitic fluid, since clinically and pathologically excitation of bilirubin formation was noted.

In only a small number of the total instances reported in the literature were autopsies made. The greater number of the cases have been reported on the basis of operations with occasional secondary operations. The extent of the involvement in these cases is therefore somewhat uncertain. Many of the growths were described as massive, filling a great portion of the abdomen, as was true in the case reported here. In all of the instances, it seems, the spread was largely confined to the abdominal cavity, distant metastases occurring with great rarity.

The histologic structure of these tumors is so variable and at times, in the different portions of the tumors, so atypical as to incite the use of many inaccurate terms in the diagnosis. It is evident, however, that the great majority have been classified as instances of spindle cell sarcoma and many as instances of distinctive angioma or angioendothelioma because of the frequent cellular arrangement about blood vessels. It seems to us that some of the illustrations that are available bear evidence of typical liposarcomatous patterns—for example, the photomicrographs accompanying the report by Sanes and Kenny.[5] It seems to us that a differentiation of this type is possible with the proper application of fat stains. The naturally abundant fat in the omentum suggests the possibility that the lipoblast is the most frequent antecedent of many of these tumors. A similar conclusion is often suggested in analyzing many of the complicated characteristics of other retroperitoneal tumors.

SUMMARY

An uncommon tumor in a man 40 years old, running a relatively brief course (nine months), resulting in bloody ascites and showing massive growth in that portion of the abdominal cavity occupied by the omentum, which it obliterated, was an instance of liposarcoma. The metastases were limited to the liver and locally to the external surfaces of the bowel. It is suggested that many of the other eighty-three instances described in the literature having similar clinical and pathologic characteristics may also have been instances of liposarcoma. The relatively early incidence suggests a possible embryonic origin of these uncommon primary tumors of the omentum.

General Review

THE PRIMARY COMPLEX (INITIAL ANATOMIC LESION IN CHILDHOOD TYPE OF TUBERCULOSIS)

A HISTORICAL STUDY

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NEW YORK

The last two decades of the past century were notable for the fundamental contributions in the domain of tuberculosis, such as the discovery of the tubercle bacillus, the nature of the defense reaction to Koch's bacillus, the mode of infection with the same bacillus and, finally, the type of lesion induced by the first infection with the acid-fast micro-organism. The last-named discovery, designated by some authors as the Ghon tubercle, is linked with the names of two men: George Küss,[1] a practicing physician in Paris, France, and Anton Ghon,[2] professor of pathology at the University of Prague, Czechoslovakia. It is remarkable that while the name of Ghon is often quoted in the literature pertaining to this question, that of Küss is ignored, particularly in the English-speaking countries. The monograph by the latter, a monumental contribution, has never been called for in the library of the New York Academy of Medicine since 1899, when it was first acquired.

In the discussion to follow an attempt will be made to report briefly the historical development of the conception of the so-called Ghon tubercle, which is of interest from the scientific as well as from the practical point of view.

Pulmonary tuberculosis in man is classified into two types: that of adult life and that of childhood. Tuberculosis of the lung of the adult was long thought to take its origin at the apex, whence it spread to other parts of the organ. More recent studies, chiefly as a result of the use of the roentgen rays, have suggested that postadolescent phthisis usually starts not at the apex but underneath the clavicle (infraclavicular infiltration) or elsewhere in the pulmonary parenchyma. The lesion may be circumscribed, being accompanied by an intense nonspecific inflammation, surrounding the tuberculous focus, or it may be confined

From the Laboratories of the Mount Sinai Hospital.

1. Küss was born in 1867 and died on Feb. 1, 1936.
2. Ghon was born in 1865 and died on April 23, 1936.

to the peribronchial or perivascular spaces. In some instances it heals spontaneously, while in others it remains active, ultimately leading to cavity formation and to dissemination of the disease, giving the picture of chronic phthisis of the adult. It is significant that in cases of this type the tracheobronchial lymph nodes remain virtually unaffected.

Incipient tuberculosis of the childhood type consists in a minute focus of pneumonia confined to the pulmonary parenchyma and in tuberculosis of the tracheobronchial (tributary) lymph nodes, evidenced by voluminous swelling, tubercle formation and caseation.

This composite lesion, parenchymal and glandular, referred to in the literature as the primary complex or as the Ghon tubercle, has served for the last three decades as a cornerstone in the problems of tuberculosis.

Villemin first noted that when "tuberculous matter" was injected subcutaneously into a guinea-pig there developed in the area of inoculation, within a short period of time, a "nodule" which soon ulcerated and never healed. This observation was confirmed by Koch, who used living tubercle bacilli. Koch noticed also that when a certain number of tubercle bacilli were introduced subcutaneously into an animal which had previously been infected with acid-fast bacilli the local ulceration that followed the reinfection healed within a short period of time. This phenomenon (Koch's phenomenon) was subsequently interpreted on an immunologic basis. According to the experiments of Calmette and Guérin and of Römer and Joseph, the originally introduced tubercle bacilli induce a state of immunity to superinfections, which can be overcome either by the use of massive doses of tubercle bacilli or by lowering the resistance of the host. Römer therefore designated the state of immunity as relative.

Krause and Willis wrote:

> There is every reason to believe that ten or even fewer virulent tubercle bacilli placed aright will lead to progressive tuberculosis in the normal guinea pig. A tuberculous, and therefore immune guinea pig will for months hold in check ten thousand times ten bacilli, and more, when these are introduced from without. The immunity of tuberculosis to a single reinfection from without may be relative as it is frequently called, but it is amazingly high.

Studies on human beings have revealed (as will be shown later in this paper) that the great majority of children between the ages of 2 and 4 years show a typical tuberculous lesion in the lung and in the tracheobronchial lymph nodes (primary complex) and that this persists throughout adult life (Opie, Puhl, Schürmann). It also has been demonstrated that the apparently healed lesion of the lymph nodes of the adult very often harbors virulent tubercle bacilli.

As extracts from tubercle bacilli failed to induce a state of immunity to superinfection in lower animals, the opinion was expressed that in tuberculosis the immunity to Koch's bacillus, though relative, exists as long as there is a state of parasitism between the germ and the host, i. e., as long as there are living tubercle bacilli within the organism—a state which, if it exists, is usually disclosed by the tuberculin skin test. Indeed, with these observations as a point of departure has come the idea that the primary infection of children with Koch's bacillus (primary complex) serves the purpose of creating a state of immunity against subsequent infections with the same micro-organism. On the other hand, it is held by many that the primary complex plays a rôle in the subsequent development of phthisis of the adult. The infection from without, it is argued, usually carries with it an insufficient number of tubercle bacilli to overcome the relative immunity; the pulmonary disease of the adult is most likely a reawakening of the disease contracted in childhood. Under the influence of some factors the relatively immune organism loses its resistance, at which time the tubercle bacilli immured within the tracheobronchial lymph nodes abandon their parasitic existence, are disseminated and set up the malady. Thus the notion is created that in a full-grown person the infection of the lungs with tubercle bacilli is as a rule endogenous, being a residue from the erstwhile childhood infection.

It is not within the scope of this paper to go into details of the controversy which these theories have aroused. For particulars, the articles by Brown, Opie, Krause and Peters, Rich and Clawson may be consulted. Suffice it to say that all the researches on the epidemiology, allergy and immunity in tuberculosis conducted for the last three decades have been but repercussions of the concept of the primary complex.

It was in the early part of the past century that physicians first noticed that the pathologic aspect of pulmonary tuberculosis of childhood is unlike that of pulmonary tuberculosis observed in adults. The views expressed by Laënnec, Andral and Cruveilhier are representative in this respect.

Laënnec, in his "Traité d'auscultation," wrote as follows:

> The tuberculous matter is more often found (in children) in the bronchial glands only and sometimes in cases in which there are no tubercles in the lungs or other serious involvements of these organs. This is particularly so in scrofulous children.

Andral stressed the rarity of tuberculosis of the lymph nodes in adults and its frequency in children. He observed that in the preadolescent period tuberculosis is by far more extensive in the bronchial lymph

glands than in the lungs and that glandular tuberculosis is usually coincident with inflammation of "respiratory channels." He also came across cases in which enormous tuberculous adenopathies were accompanied by a few minute tubercles in the pulmonary parenchyma. In many cases he found the pulmonary tubercle to be calcified and surrounded by a fibrous capsule.

Cruveilhier stated:

> A group of lymphatic glands may be tuberculous while the organ with which the glands are connected are free from tuberculosis; however, when the organ is tuberculous, the corresponding glands are nearly always involved by the same disease. Thus the bronchial lymph nodes are always more or less tuberculous in cases of pulmonary tuberculosis. But there exist numerous cases in which the tracheobronchial lymph nodes are considerably involved by tuberculous processes with no disease of the lungs or with only a few scattered tubercles in these organs. There is then no direct relationship between tuberculosis of the lymph glands and that of the corresponding organs . . . It has been definitely established that tuberculosis of the bronchial glands plays a much more important rôle in children than in adults; for example, in a child of 4, I found a large number of tuberculous bronchial glands without being able to find a single tubercle in the pulmonary parenchyma.

Parrot, in 1876, in a brief note read at a meeting of the Société de Biologie under the chairmanship of Claude Bernard, expressed his opinion as follows:

> At the ages from 1 to 7 there is no pulmonary disease which does not reflect itself distinctly in the bronchial lymph nodes which are the mirror of the lungs. Conversely, there is no bronchial adenopathy which has not its origin in the pulmonary parenchyma . . . Whenever a bronchial lymph node is the seat of a tuberculous lesion there exists an analogous lesion in the lungs (adénopathie similaire) . . . The alterations in the lymph nodes are usually more extensive than those of the corresponding pulmonary parenchyma.

Hervouët, the following year, in a thesis inspired by Parrot, reported the results of 145 necropsies in which the "primary pulmonary focus was never missing." "The lesion of the lymph nodes," he wrote, "is very conspicuous, while that of the lung is small and can be discovered after a careful search only." He found the primary pulmonary lesion in different parts of the lungs and in the vicinity of the pleura. "Invariably those glands are involved," wrote Hervouët, "which are tributaries of the tuberculous lung."

Since then the observation of the constancy of the double tuberculous lesion in infancy has been firmly established. In the French literature this phenomenon is known as the "law of Parrot" (la loi de Parrot).

George Küss, in 1898, was the first to make an exhaustive study of this subject and to interpret fully its significance. It is well to look into the methods employed by this Parisian clinician who obtained results

which have invariably been confirmed by others and to which little new has been added since. Küss wrote:

> The examination of the lungs was preceded by that of the mediastinal lymph nodes. The thoracic organs were removed en masse and the glands examined one by one, thus revealing the minutest lesion which otherwise would have escaped . . . The lungs were first thoroughly palpated, then cut with a brain knife in fine slices. In cases of failure the examination was repeated the following day . . . All the other organs were minutely examined.

Fig. 1.—George Küss, 1867-1936.

Küss was impressed by the "remarkable constancy of the law of Parrot." Several years of investigation revealed to him that:

> The pulmonary focus is extremely small in comparison with the extensive involvement of the lymph nodes. The size of the lesion in the lung is that of a pinhead, a pea or a wild cherry; it is often encysted in a fibrous shell containing caseous material; in instances of cure the lesion becomes calcareous . . . It occurs with about equal frequency in either lung . . . While its seat of predilection is the lower lobes, it is occasionally observed in the upper lobes, but scarcely ever at the apex. Its usual site is in the subpleural region, and only

occasionally is it observed within the pulmonary parenchyma . . . (fig. 2). I imagine one will not contradict me in my assertions that since one of the two lesions is up the lymphatic stream while the other is down, the first, that is, the pulmonary, should be looked on as the primary . . . It is astonishing that in Germany Weigert, Ziegler, Echhorst, Ziemssen, Strümpell and Biedert are of the opinion that, as a rule, the primitive tubercle in the infant is in the bronchial lymph nodes.

Küss was a pupil of Hutinel; Hutinel, a pupil of Parrot. It is of interest that neither Parrot nor his assistants understood the significance of their observation. Nevertheless they intuitively "stuck" to the "law," transmitting it to the next generation. Küss, the third in line after Parrot, made its meaning clear. "The law of similar mediastinal adeno-

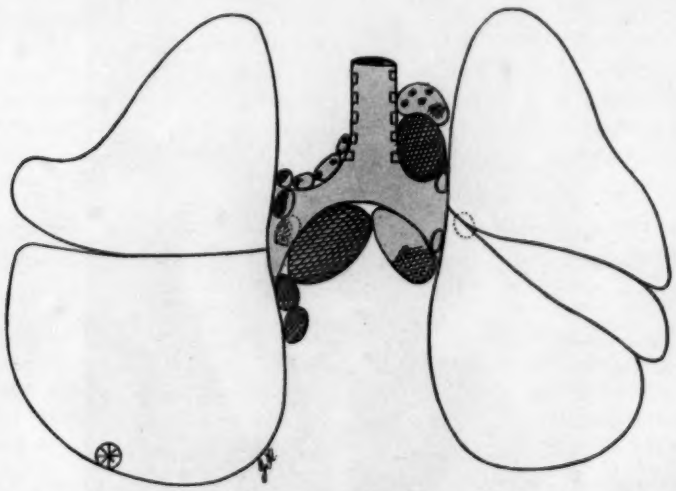

Fig. 2.—A tuberculous nodule in the left lower lobe and mediastinal adenopathy (chancre d'inoculation avec adénopathie similaire); a schematic drawing by Küss (De l'hérédité parasitaire de la tuberculose humaine, Paris, Asselin & Houzeau, 1898, fig. 7, p. 300).

pathies," he wrote, "is not only of theoretical interest but helpful in the study of the natural history of the disease and provides us with most important information on its etiology."

Since the time of Hippocrates tuberculosis in man has been looked on as a hereditary disease. Historians of tuberculosis quote Hippocrates to the effect that a "consumptive is always born from a consumptive." The noted pathologist von Baumgarten was one of the staunch supporters of this ancient view. The discussion then was centered around the mode of the alleged intra-uterine infection: whether it was paternal or maternal. The opinion was prevalent that the mother transmitted the tubercle bacilli via the placenta. The fact that very few cases of

congenital tuberculosis had been reported in the literature, while in older infants the infection with Koch's bacillus was not uncommon, was explained on the basis that in the newly born the disease passed first through a silent or occult stage (*"état de latence lymphoide"*) to become activated after a period of incubation. In support of this notion the correct observation was quoted that virulent tubercle bacilli are often found in the mediastinal lymph nodes of persons who show no tuberculous disease of visceral or other organs.

The aim of Küss in his excellent study was first of all to dispose of this antiquated hypothesis; hence the odd title of his monograph: "De l'hérédité parasitaire de la tuberculose humaine." A careful study of the few reports of cases of hereditary tuberculosis in the literature showed that they concerned advanced tuberculosis in the mothers, which was often complicated by miliary dissemination of the disease. In most of these cases the mother as well as the offspring succumbed shortly after the delivery. As the intra-uterine infection occurred through the umbilical vein, tuberculosis of the placenta was a common finding in these patients. The disease affected primarily the liver and the spleen, and the lungs became involved subsequently. In brief, as tuberculosis is usually not a septicemic disease, it is not transmitted from mother to fetus by way of the blood stream, and even when the blood of the mother is invaded by Koch's bacillus the placenta plays the rôle of an effective barrier against the passage of the germ to the fetus. Summed up: *Les enfants des phtisiques ne nâissent pas tuberculeux, mais tuberculisable* (Children of consumptives are not born tuberculous but are liable to become so).

Butel, in 1888, was the first to advocate that the invasion of the lungs by tubercle bacilli is effected via the gastro-intestinal tract. Küss said:

> It is true that ingested tubercle bacilli may pass the intestinal mucosa without producing an ulceration at the point of entry. But in these cases there invariably occurs a tuberculous involvement of the draining mesenteric lymph nodes. The tubercle bacilli that have reached the lungs from the intestines set up multiple foci in the lungs which are unlike those observed in the primary tuberculous focus.

Küss then stresses that:

> Whichever may be the portal of entry of the tubercle bacilli they leave a trace at the point of entry or at least in the neighboring lymphatic zone.

If one is to exclude heredity and ingestion as essential factors in the inauguration of pulmonary tuberculosis, what is the avenue of infection with Koch's bacillus in childhood?

Villemin, whom I have quoted, demonstrated that in the lower animal there develops an ulceration at the point of inoculation of the "tuber-

culous matter," followed shortly by tuberculization of the tributary lymph nodes. In man, too, related Küss, it was observed that when ritual circumcision was performed by a person with ulcerated tuberculosis of the lungs there occurred a tuberculous ulcer at the site of the operation, and that this was followed within two or three weeks by tuberculization of the inguinal (tributary) lymph nodes.

Küss pointed out:

> In cattle I never failed to find at autopsy a typical tuberculous tumefaction at the site where the inoculation was performed . . . In animal and man a tuberculous lesion is formed at the very point of entry of the tubercle bacilli, which, by analogy with syphilis, may be designated as the chancre of inoculation.

Küss warned, however, that the analogy does not seem at first glance to be exact, for in the guinea-pig the original lesion represents a firm nodule, which only ultimately becomes ulcerated. But in the child, too, the pulmonary lesion undergoes a similar evolution:

> At the beginning it represents a well circumscribed nodule, generally subpleural, which may remain so indefinitely. But in time the pulmonary lesion becomes ulcerated, the nodule softens, pouring out its contents into a bronchus, thus forming a purulent cavity which represents a permanent danger.

Thus, from clinical and experimental observations, Küss reached the conclusion that the composite tuberculous lesion found in infants is the point where the tubercle bacilli have come in contact with the lungs for the first time, inducing a *chancre d'inoculation avec adénopathie similaire;* also, that the micro-organism has reached the infant's lung not in utero or from the intestinal tract, as formerly believed, but by inhalation—an aerogenous infection.

As no man is a prophet in his own country, the work of Küss did not receive in France the attention that it merited.

The Austro-German pathologists were quick to grasp the far reaching importance of the study. H. Albrecht, on a large autopsy material, repeated Küss' study, corroborating it word for word. However, not until Ghon published his monograph, in 1912, did the teaching of Küss become common knowledge. The article by Ghon was so imposing as to give the impression that he was the originator of this conception; hence the misnomer "Ghon tubercle," used by Clawson and others.

In justice to Ghon it must be stated that he gave credit to Küss for this remarkable observation. Thus, in the introduction to his monograph, published in 1912, he stated: "We are indebted to Küss for a thorough discussion of the law of Parrot in tuberculosis; the question of the portal of entry of tubercle bacilli in infancy was detailed by him in a most comprehensive manner." In the preface to the English translation of the monograph, which appeared in 1916, he wrote: "The

results of my investigation confirm the evidence of Küss and of H. Albrecht. They do not really state any new facts."[3]

The last twenty years of Ghon's life have been largely devoted to the study of the primary complex—to use the term introduced by Ranke for the *chancre d'inoculation avec adénopathie similaire*. In articles published in recent years he has again reaffirmed the original findings of Küss. Thus he wrote:

> In cases of erstwhile infection with the tubercle bacillus there always occurs locally a primary focus whether it concerns the lungs, the bowel, the nose or the skin. Invariably the infection is primarily local, which sooner or later will cause a similar involvement of the regional lymh nodes, giving the characteristic picture of the "Primary Complex."

Opie studied the erstwhile tuberculous lesion in the lungs of adults. He found that it occurs in at least 92 per cent of all adults and is identical with the childhood type of tuberculosis.

Puhl, in a study inspired by Aschoff, investigated in adults the macroscopic and microscopic appearance of the remnants of the initial childhood infection. He found such remnants to be nearly always present (in 90 per cent of 122 patients). He attempted to ascertain whether it is possible to differentiate between the erstwhile infection of childhood and the subsequent reinfection of the adult on the basis of the morphologic aspect. His conclusion was that the two lesions are respectively distinctly different: The erstwhile tubercle heals by calcification and ossification, a process which is rarely observed in the adult type of infection; the original lesion caused by infection in childhood is invariably under the pleura, while the infection of the adult occurs at the apex within the pulmonary parenchyma "so that when it heals it causes contraction of the surface of the lung in the process of cicatrization." But what is particularly important is the structure of the capsule surrounding the primary complex, which is made up of an inner specific layer and an outer nonspecific one. In the *Reinfekt* the capsule is remarkably thin. Puhl's contention is that the scar resulting from the healed tuberculous infection of the adult is characteristic in that it shows that the infection resulted not from the allegedly dormant childhood lesion but from a new infection from without.

While epidemiological and clinical studies conducted in recent years (Brown, Opie) are to the effect that adult phthisis is probably often of exogenous origin, convincing microscopic evidences in favor of this conception are lacking.

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3. Hedrén (Ztschr. f. Hyg. u. Infektionskr. **73**:273, 1912-1913), Lange (Ztschr. f. Tuberk. **38**:167 and 263, 1923) and others merely kept reiterating and elaborating Küss' thesis.

Ranke, who introduced the term "primary complex," compared the erstwhile tuberculous infection to the primary infection in syphilis: Like syphilis, tuberculosis was divisible into three phases, the first being that of the primary complex, the second that of hypersensitiveness and of generalization of the allergy to the germ and the third that of relative immunity accompanied by localized infection, i. e., chronic phthisis of the adult. Ranke, then, regarded postadolescent phthisis as being usually a residue from the primary focus of the childhood infection.

As the work of Küss was carried on before the advent of immunology there is no mention in his monograph of the possible immunizing properties of the erstwhile infection. However, he stressed the point that the childhood tuberculous lesion may serve as a source for autoinfection in later periods of life. Thus he wrote:

> One of the most frequent causes of phthisis is the existence in the mediastinum of latent caseous foci which the individual harbors since infancy . . . A certain number of cases of tuberculosis of childhood, of adolescence and even of adults goes back to the latent foci which appeared in early life.

SUMMARY

Pasteur once made the statement that: "Without theory practice is but routine born of habit. Theory alone can bring forth and develop the spirit of invention . . . Do not share the opinion of those narrow minds who disdain everything in science which has not an immediate application." The history of the conception of the primary complex is a case in point. When Parrot first related his findings on the composite tuberculous lesion of childhood, in 1876, their significance was not appreciated by him or by his contemporaries; the observation was looked on as merely theoretical. However, repeated experience revealed the great practical importance of this discovery.

Küss, a practicing French physician, in 1898 first discredited the belief held by his contemporaries that tuberculosis is a hereditary disease transmitted from mother to fetus via the placenta. He then produced convincing evidence to show that the disease is acquired as a rule post partum by inhalation of Koch's bacilli—an aerogenous infection. He further ascertained the type, the constancy and the pathognomonic character of the primitive lesion (*le foyer pulmonaire primitive*), which he described as a minute subpleural tuberculous granuloma accompanied by tuberculosis of the tracheobronchial lymph nodes. This he designated as *chancre d'inoculation avec adénopathie similaire*. The same phenomenon was subsequently defined as the primary complex; also as the Ghon tubercle.

The concept of the primary complex as expressed by Küss is looked on as a fundamental observation which has served as a basis for all

research pertaining to the problems of tuberculosis, such as the tuberculosis of childhood, the spread of the disease, immunity and allergy, conducted for the last three decades.

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Obituaries

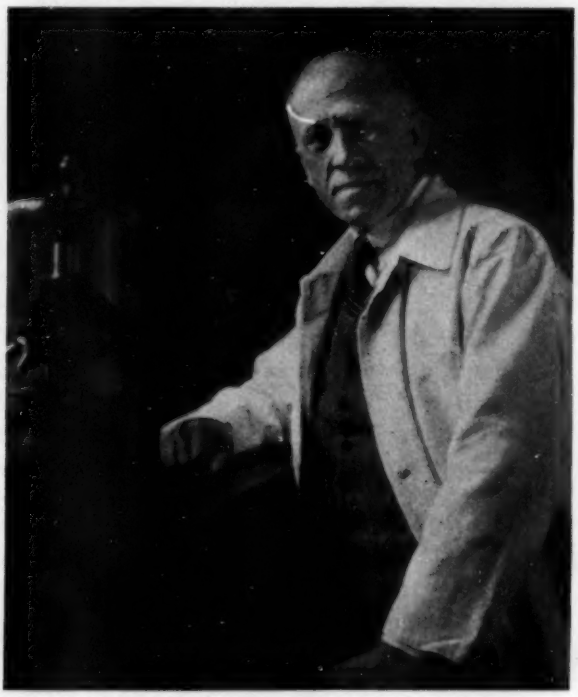
OSKAR KLOTZ

1878-1936

Oskar Klotz, Toronto, Ontario, M.B.(Tor.), M.D.C.M.(McGill), F.R.C.P.(C), F.R.S.(C), son of the late Dr. Otto J. Klotz, director of the Dominion Observatories, Ottawa, was born at Preston, Ontario, Jan. 21, 1878. His early education was at the Galt and Ottawa Collegiate Institutes. He received the degree of Bachelor of Medicine from the University of Toronto in 1902 and the degree of Doctor of Medicine and Master of Surgery from McGill University in 1906. He pursued postgraduate studies at McGill University, Montreal, and in Germany at the University of Bonn, in 1905; at the University of Freiberg in 1908, and at the University of Marburg in 1914. He was house physician to the Ottawa General Hospital in 1902 and superintendent of the Ottawa Civic Hospital for part of 1903. He became the Governor's fellow in pathology at McGill University in 1903-1905, a fellow in pathology from the Rockefeller Institute in 1905-1906, demonstrator in pathology and bacteriology at McGill University in 1905-1907, assistant pathologist at the Royal Victoria Hospital and pathologist at the Maternity and Alexandra Hospitals, Montreal, in 1905-1909 and lecturer in pathology at McGill University in 1907-1909. In the autumn of 1909 he went to the University of Pittsburgh as professor of pathology and bacteriology, a position which he held until the end of 1920, and during this period he was director of the laboratories of the Mercy Hospital, director of the pathology laboratory of Magee Hospital, and in 1916-1919 consulting pathologist to the United States Bureau of Mines and the United States Department of War at Washington. The eleven years in Pittsburgh were important not only in his own development but also in establishing pathology in that community. He was professor of pathology in the Faculdade de Medicina, São Paulo, Brazil, from 1921 to 1923, having been appointed by the International Health Board of the Rockefeller Foundation. He carried with him to Brazil the very best of North American pathology and left a deep impression on his colleagues there. He returned from South America in 1923 to fill the position of professor of pathology and bacteriology in the University of Toronto, his alma mater, and he also became director of the pathology laboratories of the Toronto General Hospital, consulting pathologist to the Hospital for Sick Children and in 1934 honorary consultant in pathology to the Department of Health of

Ontario. During these years in Toronto he had working with him many graduates from Pittsburgh and from Brazil. The latter were sent by the Rockefeller Foundation and included Dr. Cunha Motta, 1924-1925; Dr. A. de G. Magalhaes, 1929-1930, and Dr. C. Magarinos Torres, 1930-1931.

He held a number of positions in various commissions, councils and societies; he was, for instance: a member of the associate committee on tuberculosis from 1925 and a member of council from 1932 in the



OSKAR KLOTZ
1878-1936

National Research Council of Canada; a special member of the yellow fever commission of the International Health Board of the Rockefeller Foundation in 1926 and 1928 (he spent six months of these years in Nigeria, West Africa, as a result of which, on his return voyage in 1928, he suffered a severe attack of tropical malaria); joint chairman of the committee for Canada of the International Society of Geographical Pathology from 1929 (he was one of the two representatives for Canada at the meeting in Utrecht, Holland, in 1934—the topic of the symposium being arteriosclerosis); a member of the Ontario branch of

the St. John Ambulance Association in 1933 and a member of council from 1935; a member of the Toronto Health League in 1933; a member of the Cancer Commission of Ontario and chairman of its subcommittee on Research from 1934; a member of the medical advisory committee in conjunction with the Ontario Society for Crippled Children from 1935; an honorary life member of the Hamilton Health Association in 1936.

He was in charge from 1918 of the reviews in the department of pathology and bacteriology for the progress of medical science of the *American Journal of the Medical Sciences.*

The scientific societies to which he belonged, in many of which he held office, were: Academy of Medicine, Toronto (president, 1935-1936); American Association for the History of Medicine (councilor, 1935); American Association of Pathologists and Bacteriologists (president, 1919); American College of Physicians (Fellow, 1924); American Society of Parasitologists; American Society of Tropical Medicine; Association of American Physicians; Association of Medical Museums (president, 1919); Canadian Medical Association; International Society for the History of Medicine; Ontario College of Physicians and Surgeons; Ontario Medical Association; Pathological Society of Great Britain and Ireland; Royal Canadian Institute (councillor, 1935); Royal College of Physicians and Surgeons of Canada (fellow, 1931); Royal Society of Canada (fellow, 1928); Society for Experimental Biology and Medicine; Society for Experimental Pathology (councilor, 1932; president, 1935); Society for Tropical Diseases (corresponding member).

He was a member of the York Club, Toronto, and of the Arts and Letters Club of Toronto. He belonged to the Zeta Psi, Alpha Omega Alpha and Nu Sigma Nu fraternities.

He was married to Stella M. Scovil, daughter of the late Mr. T. K. Scovil, of Portland, Ontario, on March 4, 1908. There were no children.

His work covered the whole range of pathology and bacteriology, including reports on diseases of the lungs, liver and kidneys, on such general diseases as rheumatism, tuberculosis, influenza and cancer, on pathologic technic and on general pathology, but he is most widely known for his work on arteriosclerosis and other diseases of the arteries, which was one of his most active interests throughout all of his scientific career. His earliest work dealt with the experimental production of arteriosclerosis, and his later extended investigations of the process of calcification were a direct outgrowth of this interest in diseases of the arteries. These studies led to the formulation of the theory of pathologic calcification with which his name is now generally associated.

Further experimental studies of arteriosclerosis followed. Although he was an ardent opponent of Thoma's theory of medial weakening and compensatory hyperplasia of the intima, he himself was much interested in diseases of the media of arteries and was largely responsible for the introduction of the concept of "medial arteriosclerosis." Diseases of the media formed the subject of a long monograph published in 1911, while later publications dealt in further detail with the medial lesions associated with rheumatic and syphilitic infections and the changes responsible for the occurrence of aneurysms and spontaneous ruptures of the aorta. Arteriosclerotic lesions affecting the intima likewise attracted his attention. He was especially interested in sclerosis of the coronary and renal arteries and made numerous contributions to the literature of coronary sclerosis and cardiovascular renal disease. His experimental studies of earlier years strongly influenced his ideas on the etiology of arteriosclerosis, and he constantly supported the view that infections, intoxications and overwork with consequent fatigue of the arterial musculature were etiologic factors of first importance. His masterly discussion of arteriosclerosis before the meeting of the American Association of Pathologists and Bacteriologists in Toronto in 1934 and the exhaustive review of experimental arteriosclerosis presented before the International Society of Geographical Pathology in Utrecht in the same year formed together a fitting climax to a life-time of research on diseases of the arteries.

During the last fifteen years of his life he was attracted by the problems of tropical diseases, particularly those of yellow fever. During his stay in São Paulo, Brazil, in 1921-1923, and as a special member of the yellow fever commission of the International Health Board of the Rockefeller Foundation in Nigeria, West Africa, in 1926 and in 1928, he came in intimate contact with all phases of yellow fever and took a prominent part in its scientific study. He was probably the leading authority on the pathologic diagnosis of the disease in man and animals, and the evidence he collected established the identity of the yellow fever of West Africa with that occurring in America. The West African studies eliminated as causative agents any visible microorganisms, and an endeavor was made to discover a susceptible animal other than man for experimental purposes. The attempt to find such an animal in West Africa having failed, his suggestion that wild animals from countries free from yellow fever be investigated led to the brilliant results which established the virus nature of the disease.

He was associated with a number of great men in medicine: Adami, John McCrae, Simon Flexner, H. Ribbert, H. Chiari, Jores, Bernard Fischer, Aschoff and others.

Dr. Klotz was a really great teacher, inculcating in his students enthusiasm for the principles of scientific study and an understanding of the importance of the historical background and always making clear the probable nature of future progress. His laboratories were filled with eager research workers, and these he inspired by his unrelinquishing demand for exact, honest and unprejudiced study as well as for precise records of all phases of their work. His own laboratory records are models of accuracy, completeness and keen observation. He expected, as he gave, the best of man's qualities. He was ever alert to new ideas, and the enthusiastic suggestions of the younger men received his sympathetic attention but he was intolerant with those who were not serious in their dealings with him.

Being himself unsparingly energetic, decisive and clear-thinking, he gave to all that sense of security and definiteness of purpose so essential in the chief of a scientific department. His executive ability would have assured a brilliant success in many lines of endeavor, and he was often called on to help in the organization and clarification of many problems outside his own particular field.

All the past and present members of his staff knew that they had in Dr. Klotz a fatherly advisor not only in things related to their work but in all matters pertaining to their general welfare. He gave unsparingly of his time and was always encouraging and helpful. He usually insisted that his associates publish the outcome of their studies under their own names, and thus a great deal of the work from his laboratories appears without his name, although he guided, controlled and was familiar with it all. The museum of pathology of the Banting Institute, filling an entire floor, is unexcelled for teaching purposes, and the specimens and slides used by the students in their studies represent a lifetime of discriminating selection.

He was fond of the outdoors; in his younger days he was a skilful canoeist, and always a lover of nature—of trees, flowers, birds and all wild life, being fully happy wandering through great woods with his keen mind all attention for new discoveries. His summer home, "Silverwoods," on the Rideau, near Portland, is a charming place of many acres of rich wooded land and glorious sweeps of lake and sky. His bungalow is decorated with unusual things collected in South America and West Africa. Here was the place where Dr. Klotz found relaxation, but it was only a change of his characteristic activity, which was necessarily so in one of his temperament. He delighted in planning and constructing a new boat house, a guest house, a laboratory in the woods and all the details which made this such a lovely refuge. Those who were his guests here found him as always a delightful and generous host.

The richness of his experiences and the wide and detailed knowledge which he had accumulated in a life of unusual fulness are only to a very small degree retained for his followers in well over a hundred important scientific papers as well as a long list of masterly addresses on a great variety of subjects. The loss to medical science of one in the very prime of his productivity is indeed a tragedy, but he will long live through the splendid influence which he exercised on all with whom he came in contact. He had been in failing health for over two years with myelogenous leukemia, but the disease had little effect on his mental energy, and he worked vigorously until the end, which came with a minimum of physical suffering on November 3.

The poem "Recompense" written by his dear friend the late Dr. John McCrae seems an appropriate remembrance:

I saw two sowers in Life's field at morn,
To whom came one in angel guise and said,
"Is it for labour that a man is born?
"Lo: I am Ease, Come ye and eat my bread!
Then gladly one forsook his task undone
And with the Tempter went his slothful way,
The other toiled until the setting sun
With stealing shadows blurred the dusty day.

Ere harvest time, upon earth's peaceful breast
Each laid him down among the unreaping dead.
"Labour hath other recompense than rest,
Else were the toiler like the fool," I said;
"God meteth him not less, but rather more
Because he sowed and others reaped his store."

W. L. HOLMAN.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Experimental Diabetes and Tuberculosis in the Dog. M. M. Steinbach, S. J. Klein and M. Deskowitz, Am. Rev. Tuberc. **32**:665, 1935.

From experiments it is concluded that the dog rendered diabetic by pancreatectomy becomes vastly more susceptible to infection by tubercle bacilli and that this susceptibility is due not so much to lack of the pancreatic digestive enzymes and the consequent emaciation as to the faulty metabolism of carbohydrate.

H. J. Corper.

The Relation of Disease of the Liver to Anemia. M. M. Wintrobe, Arch. Int. Med. **57**:289, 1936.

Except when hemorrhage or a complicating infection was associated with it, anemia associated with hepatic disease was either normocytic or macrocytic. The macrocytic anemia was morphologically similar to, if not identical with, that seen in pernicious anemia. Like the latter, it manifested spontaneous remissions and was influenced by intramuscular liver therapy. Macrocytic anemia was found with hepatic disease of some duration and of wide extent. It was most common with cirrhosis of the liver. It was not found with acute necrosis of the liver or when the damage to the liver was only slight or moderate in severity. Foci of blood formation in the spleen were observed in the presence of hepatic disease with macrocytic anemia as well as with other forms of macrocytic anemia, but with two exceptions they were not noted in association with any other type of anemia. The observations recorded support the hypothesis that macrocytic anemia in the presence of hepatic disease results from an inability to store a necessary hematopoietic principle.

From the Author's Summary.

Tumor of the Pineal Body. Gilbert Horrax, Arch. Neurol. & Psychiat. **35**: 215, 1936.

On the basis of his observations on two patients with tumor of the pineal body Horrax contends plausibly that the pineal body is concerned with the regulation of secondary sex characteristics. One of the patients, a boy aged 10, appeared to be aged 16 and within one year showed great increase in height and weight, an abundance of bodily hair and an unusually well developed musculature, with an adult appearance of the external genitalia and a masculine voice. A simple decompression followed by roentgen treatment caused marked change in this boy, who became more youthful, but there were no changes in the voice or in the size of the genitals.

George B. Hassin.

Changes in the Aorta in Experimental Open Pyopneumothorax. Fanny Chaletzkaja, Virchows Arch. f. path. Anat. **295**:245, 1935.

The author describes the histologic changes in the rabbit's aorta after the production of open pyopneumothorax. On the second or third day areas of necrosis are evident in the media. By the fifth or sixth day each necrotic area has become surrounded by a zone of cellular inflammatory reaction which extends into the intima. At still later stages the necroses are surrounded by newly formed connective tissue and the covering intima is thickened. The changes described occur only where the aorta is separated from the inflamed pleura by a thin layer of connective tissue. Where the aorta is surrounded by a layer of adipose tissue no alterations are observed.

O. T. Schultz.

RENAL EXCRETION OF DYES. G. EKEHORN, Virchows Arch. f. path. Anat. **295**:256, 1935.

Ekehorn had previously criticized deductions relative to the formation of urine drawn by Sheehan from the disappearance of injected substances as determined by simultaneous quantitative estimations on renal arterial and venous blood. The method was held to be especially faulty when applied to injected dyes because the degree of disappearance of the dye from the venous blood is greater than can be accounted for by the amount of glomerular filtrate formed. The controversy thus begun was continued by Sheehan. In the present contribution of thirty-four pages Ekehorn continues the debate. He maintains his position and insists that the method used by Sheehan is faulty in that it does not take into account the diffusion of substances into the renal tissue and that the method must be "judiciously applied" if the results obtained by it are to be used in the interpretation of matters concerned with tubular resorption.

O. T. SCHULTZ.

Pathologic Anatomy

BLOOD VESSELS IN HUMAN HEART VALVES. T. WEARN, A. W. BROMER and L. J. ZSCHIESCHE, Am. Heart J. **11**:22, 1936.

One hundred hearts from patients without history or clinical evidence of endocarditis were examined for blood vessels in the valves, which were demonstrated in eighty-six. Twelve of the hearts showed evidence of active or healed inflammation of one or more valves. Active or healed valvulitis occurred in the absence of blood vessels, and blood vessels were found in valves without evidence of active or healed valvulitis. Of the eighty-eight hearts without evidence of previous inflammation, 84 per cent showed blood vessels in one or more of the valves. The incidence of blood vessels in a given valve was not in keeping with the incidence of rheumatic valvulitis in that valve. A method of making an injection into the blood vessels in cardiac valves is described.

FROM THE AUTHORS' SUMMARY.

WEIGHT OF THE THYMUS AND ITS COMPONENT PARTS AND NUMBER OF HASSALL CORPUSCLES IN HEALTH AND IN DISEASE. EDITH BOYD, Am. J. Dis. Child. **51**:313, 1936.

Norms of the involutional changes and of the variations in weights and in numbers of Hassall corpuscles with age have been constructed for the parts of the thymus similar to those for the whole gland from Hammar's data on 207 cases of death from accidental causes within twenty-four hours after injury. These norms show that only the medulla, with its Hassall corpuscles, begins, like the whole thymus, to involute at about the age of puberty; involution of the lymphoid tissue in the cortex starts at 4 years of age, while the connective tissue and fat continue to increase until old age. Comparison of the weights of the thymus and its parts and of the numbers of Hassall corpuscles in cases of death from disease with the norms shows that (1) within from one to seven days fortuitous involution has progressed far enough to reduce the weight of the parenchyma and cortex markedly and that of the medulla slightly without affecting the number of Hassall corpuscles except when it is temporarily increased because of acute infection, and (2) within one week or more it has progressed sufficiently to reduce markedly the number of Hassall corpuscles and the weight of the connective tissue and fat except in persons with exophthalmic goiter, in whom the parenchymatous structures are hypertrophied and the connective tissue and fat are unchanged. The frequency of the occurrence of fortuitous involution and the rapidity of its onset explain why the normal thymus is rarely seen except in cases of sudden death.

FROM THE AUTHOR'S SUMMARY.

AN UNDESCRIBED GRANULE WITHIN THE LYMPHOCYTE. E. A. GALL, Am. J. M. Sc. **191**:380, 1936.

A description is given of certain motile refractile globules which are normally present in lymphocytes of the peripheral blood, observed in wet preparations. The bodies vary from point size to about 0.5 micron in diameter. They appear in approximately 34 per cent of the circulating lymphocytes, for the most part in normal lymphocytes though they have been noted in pathologic cells as well. The number of granule-bearing lymphocytes remains quite constant in the same person from day to day. They do not appear spontaneously or disappear in the standing blood spread. One or two are observed in a cell, rarely more. The spherule is not mitochondrial nor is it one of the bodies staining with neutral red previously described as existing in lymphocytes. It is probably not of a lipoid nature and not a portion of the centrosphere. It appears to be a hitherto undescribed constituent of the cytoplasm of the lymphocyte. FROM THE AUTHOR'S SUMMARY.

MORPHOLOGY OF THE ADRENAL CORTEX. R. L. ZWEMER, Am. J. Path. **12**:107, 1936.

The morphology of the adrenal cortex seems to reflect the demands imposed by body needs. An excess of hormone over normal requirements is shown by an increase in the number of storage cells. An acute demand causes discharge of the mature cells but does not immediately affect cells of the accumulative type. Prolonged demands stimulate the formation of new cells, a process which may or may not be able to counteract the utilization of cells in the discharging phase.

FROM THE AUTHOR'S SUMMARY.

TREPONEMA PALLIDUM IN SYPHILITIC AORTIC VALVULITIS. A. B. RICHTER, Am. J. Path. **12**:129, 1936.

The case reported is the twenty-first authentic instance of syphilitic endocarditis of heart valves to be recorded in the literature. It is the ninth case of such involvement of the aortic valve. The process in this valve had extended from the root of the aorta into the posterior cusp. An acute exacerbation in the distal portion of the cusp produced subacute syphilitic endocarditis. Spirochetes were present in the cusp and valve ring. No record of a previous demonstration of spirochetes in a cardiac valve was found. Congenital bicuspid aortic valve and subaortic stenosis, two rarely associated cardiac anomalies, were also present. The subaortic stenosis produced signs of aortic stenosis in the anatomic presence of aortic insufficiency, leading to the diagnosis of rheumatic aortic stenosis and insufficiency. FROM THE AUTHOR'S SUMMARY.

LIVER REGENERATION AND BILIARY OBSTRUCTION. G. R. CAMERON, J. Path. & Bact. **41**:283, 1935.

Obstruction of the bile outflow from the liver in rats so alters the liver cells that they are unable to grow when transplanted. Bile ducts on the contrary are unaffected. FROM THE AUTHOR'S SUMMARY.

HISTOLOGIC CHANGES IN RATS GIVEN INJECTIONS OF ESTROGENIC AND OF TESTICULAR HORMONE. V. KORENCHEVSKY and M. DENNISON, J. Path. & Bact. **41**:323, 1935.

Two effects produced by the estrogenic hormone in castrated rats appear to be physiologic and were seen in the seminal vesicles and coagulating gland and to a lesser degree in the prostate: *(a)* a stimulation of the development of smooth muscle; *(b)* an enhancement of the effect produced by testicular hormone. These effects were slight as compared with those obtained with large doses of testicular

hormone alone. Injections of the estrogenic hormone also produced a partial return toward the normal histologic structure of the so-called castration hypophysis. Of the pathologic effects of the estrogenic hormone the most important are a fibrosis and epithelial metaplasia produced in some of the secondary sex organs of the castrated rat and a hypertrophy of the adrenals and hypophysis of the normal rat. In particular the following changes in the prostrate of the castrated rat are produced: in all lobes, a slight increase in the size of the epithelial tubules and in the amount of secretion; in the dorsal lobe, a hyperplasia and metaplasia of the epithelial cells converting a number of tubules into nearly solid cords; chiefly in the dorsal and central lobes, an increase of smooth muscle and to a greater extent of fibrous tissue. The administration of testicular hormone simultaneously with the estrogenic hormone to castrated rats almost completely prevented these changes in the secondary sex organs, nor were these changes present to a significant extent in normal rats possessing natural testicular hormone when these rats were given injections of estrogenic hormone.

FROM THE AUTHORS' SUMMARY.

CHANGES IN INFERIOR LARYNGEAL NERVES IN TUBERCULOUS LARYNGITIS. B. I. LAURENTIEV and A. G. FILATOVA, Trav. du lab. de recherches biol. de l'Univ. de Madrid **29**:319, 1934.

In the course of tuberculosis of the larynx changes in the inferior laryngeal nerves often develop, beginning in the terminal apparatus. The initial stage is marked by swelling of the nerve fiber and its ramifications by an excessively rapid growth of the nerve filaments, by formation of clubs and crescents, and by massive thickening, followed by atrophy and destruction of the nerve elements and muscle fibers.

CYRIL B. COURVILLE.

RECTOVAGINAL ENDOMETRIOSIS. J. J. CHYDENIUS, Arb. a. d. path. Inst. d. Univ. Helsingfors **8**:153, 1935.

Three interesting cases of endometriosis are reported in detail, two of them involving the rectovaginal septum. It is emphasized that at times endometriosis may grow more or less like a malignant tumor. It is of interest to recall that growth in endometriosis is dependent on ovarian hormones. After castration connective tissue may replace large parts of endometrioid foci which previously tended to infiltrate the surrounding tissue.

THE TISSUE REACTION IN RENAL LIPOMATOSIS. K. J. SSINELŠČIKOWA, Centralbl. f. allg. Path. u. path. Anat. **63**:49, 1935.

Two kidneys were studied. One was from the body of a man 42 years old who was cachectic, died of carcinoma of the stomach, and had ascending pyelonephritis and a stone in the left kidney. The other kidney was from the body of a woman who died of nephritis and had a complete replacement of the right kidney by fat with a stone in this and a second stone in the lower third of the ureter. This kidney had grown together with the ascending colon so that the renal stone could be felt and seen from the lumen of the bowel. After extensive microscopic studies the author concludes as follows:

Renal lipomatosis is a rare disease produced by renal stones. It is initiated and accompanied by a chronic productive inflammation. Malnutrition and parenchymatous death occur, with eventual atypical regeneration of renal epithelium. The fat is formed from local mesenchymal elements, which resorb the renal parenchyma and then become fatty. This process depends apparently on a chronic carbon dioxide hunger of all renal elements and a functional disorder and decrease in these elements with a decrease in nutrition and urinary excretion but without the participation of micro-organisms.

GEORGE RUKSTINAT.

EXTENSIVE DIAPHRAGM FORMATION IN THE RIGHT AURICLE. A. RUGGIERI, Centralbl. f. allg. Path. u. path. Anat. **63**:129, 1935.

An unusually large diaphragm was found in the right auricle of a woman aged 42 years who periodically had had cardiac decompensation and had died of cerebrospinal syphilis. The heart was 16 cm. across and 9.5 cm. vertically; the right auricle and ventricle were about twice their usual size, and the right auricular wall was 6 mm. thick. The pulmonary artery was only 5.5 cm. in circumference; the aorta, 8 cm. The diaphragm began at the mouth of the superior vena cava, retracted from the lower end of the interauricular septum, permitting passage of the little finger between its edge and the interauricular septum, and then attached to the auricular wall 1 cm. craniad to the middle tricuspid leaflet. This insertion was continuous with that on the side and beginning of the front wall of the auricle. After encircling the mouths of the coronary sinus and inferior vena cava this membrane ended in a sharp strandlike prolongation on the auricular side wall. The upper and anterior sides of this partition ended in sharp free borders, and the inferior portion was studded with fenestrations up to 1 cm. in diameter. The membrane, in general, was 1 mm. thick and composed of connective tissue in which were embedded strands of muscle up to 3 mm. wide.

The auricle contained no thebesian or eustachian valves. The anomaly was explained by an abnormal location of the inferior vena cava which prevented a fusion of the primary and secondary septums of the interauricular partition.

GEORGE RUKSTINAT.

Microbiology and Parasitology

INTRACEREBRAL INOCULATION OF FETAL GUINEA PIGS WITH BACILLE CALMETTE-GUÉRIN AND THE H37 STRAIN OF THE TUBERCLE BACILLUS. I. S. NEIMAN and O. C. WOOLPERT, Am. J. Path. **12**:153, 1936.

Guinea-pig fetuses were inoculated with graded doses of BCG and the effects compared with those obtained in a similar series inoculated with virulent tubercle bacilli (H37). Both types of tubercle bacilli were found to spread from the site of inoculation but not with equal rapidity; with the same dose, it took a longer time for BCG to cause as much pathologic change in the animal as a whole as did H37. Pathologically the response of the fetus to both organisms was the same. Histologic evidence is brought forward to show that BCG is capable of multiplying in fetal tissues and of initiating a disease process different only in degree from that produced by H37. So far, attempts at fetus to fetus transfer of BCG have not been successful. Recovery of this bacillus from inoculated fetuses by cultivation on artificial mediums was accomplished four times in twenty-four attempts.

FROM THE AUTHORS' SUMMARY.

COCCIDIOIDAL GRANULOMA: COMPARISON OF THE NORTH AND SOUTH AMERICAN DISEASES. J. W. JORDAN and F. D. WEIDMAN, Arch. Dermat. & Syph. **33**: 31, 1936.

Coccidioides immitis is firmly established as the cause of coccidioidal granuloma in most, if not all, the cases in North America at present recorded. It also was the cause in two cases in South America (Argentine). A radically different fungus, Paracoccidioides brasiliensis, is the cause of a disease which heretofore also has been regarded as coccidioidal granuloma, numerous cases of which have occurred in Brazil. We secured two strains of the latter fungus and compared them with two North American strains of C. immitis. They proved to be radically different in culture tubes and under the microscope and as to pathogenicity for laboratory animals. Only in tissue was there any resemblance between the two parasites, yet even there certain differences could be established. We believe that P. brasiliensis constitutes a new species. In view of the widely differing character

of the diseases produced by the two species, it is doubtful whether the disease produced by P. brasiliensis should be included in the category of coccidioidal granuloma. It is recommended that the name "Almeida's disease" be applied to the maladies caused by P. brasiliensis. Previous reports of North American cases of coccidioidal granuloma should be reexamined in the full light of what is known of Almeida's disease. Paracoccidioidal granuloma is a third fungous disease which must be added to blastomycosis and coccidioidal granuloma when the problem arises of differentially diagnosing blastomycosis or coccidioidal granuloma either clinically or histologically. FROM THE AUTHORS' SUMMARY.

THE DISTRIBUTION OF THE VIRUS OF LYMPHOCYTIC CHORIOMENINGITIS IN THE ORGANS OF INOCULATED MONKEYS. C. ARMSTRONG, J. G. WOOLEY and R. H. ONSTOTT, Pub. Health Rep. **51**:298, 1936.

Monkeys experimentally infected with the virus of choriomeningitis were shown by subinoculation of mice to harbor the virus in large amounts in all the organs and tissues tested, namely: adrenal glands, cerebrum, blood, kidneys, liver, lungs, lymph glands, marrow, heart muscle, voluntary muscle, pancreas, spinal cord, spinal fluid, spleen, submaxillary glands and testicle. This distribution of virus is not explainable by the presence of virus-containing blood in the vessels.

FROM THE AUTHORS' SUMMARY.

THE PATHOLOGIC HISTOLOGY OF LYMPHOCYTIC CHORIOMENINGITIS IN MONKEYS. R. D. LILLIE, Pub. Health Rep. **51**:303, 1936.

Lymphocytic choriomeningitis in monkeys is characterized by an almost constant irregularly distributed pronounced lymphocytic infiltration of the choroid plexuses sometimes accompanied by serocellular exudation into the cerebral ventricles and by an almost constant irregularly distributed lymphocytic infiltration of the leptomeninges, usually moderate, as well as by very few foci of cellular gliosis and of lymphocyte infiltration of vessel sheaths in the brain and cord. The meningeal and plexus infiltration may persist for long periods after infection. Focal lymphocytic infiltration and sheath cell proliferation occur in the spinal root ganglions.

The lungs often present congestion, serous exudation, interstitial edema, hemorrhages and perivascular lymphocytic infiltration. Pyelitis and sometimes hemorrhagic cystitis occur in a number of animals and are characterized by focal and diffuse mucosal lymphocytic infiltration and edema. Foci of coagulative to fibrinoid hemorrhagic necrosis in the liver are seen in about one fourth of the animals, and focal necroses also occur occasionally in the adrenal and parathyroid glands. Splenic congestion, a variable grade of hyperplasia of the marrow and of lymph node follicles and sinus reticulo-endotheliosis are other frequent findings. Focal interstitial or perivascular lymphocytic infiltration is frequent in the kidneys, epididymis, uterus, tube, parathyroid glands, heart, lungs and tracheal mucosa, and occasionally in the esophageal mucosa, pancreas, adrenal glands, testis, ovary and skeletal muscles. FROM THE AUTHOR'S SUMMARY.

THE TYPHUS GROUP IN MALAYA. R. LEWTHWAITE and S. R. SAVOOR, Brit. J. Exper. Path. **27**:1 and 23, 1936.

The experimental data secured indicate that the rabbit is the laboratory animal of choice for work on rural tropical typhus and the tsutsugamushi group of fevers, provided that intra-ocular inoculation is practiced. Further, the data stress the remarkable similarity of the behavior of the viruses of these two diseases in experimental laboratory animals—a similarity that is fully supported by cross-immunity experiments.

On the other hand, the guinea-pig is the animal of choice for the study of urban tropical typhus. FROM THE AUTHORS' SUMMARIES.

INFECTION OF MONKEYS (MACACA MULATTA) AND GUINEA PIGS WITH THE VIRUS OF EQUINE ENCEPHALOMYELITIS. E. W. HURST, J. Path. & Bact. **42**:271, 1936.

The infection of guinea-pigs or rhesus monkeys with the virus of equine encephalomyelitis may be divided into the visceral and the nervous phase. In the guinea-pig the two phases tend to overlap; in the monkey they tend to be separated by an interval of several days. The nervous phase succeeds the visceral much less constantly in the monkey than in the guinea-pig. During the visceral phase virus circulates in the blood in greater amount than can be accounted for readily by its titer in the viscera, while at a site of inoculation in muscle little or no multiplication takes place and in the dermis only moderate local increase. It seems, therefore, that the virus must multiply chiefly either in the blood or in some tissue intimately connected with the blood stream and forming only a small part by weight of the various viscera. In the monkey, infection of the nervous system occurs at a time when the very prompt antibody response to the visceral infection is as complete as it ever will be; monkeys recovered from the visceral infection without obvious nerve involvement are not usually immune to intracerebral inoculation of fresh virus. On the other hand, guinea-pigs recovered without having shown nervous symptoms are immune to subsequent intracerebral inoculation. Unlike many neurotropic viruses, the virus of equine encephalomyelitis inoculated intramuscularly or intradermally does not reach the central nervous system by way of the local peripheral nerves. In many cases the findings suggest that the nervous system is infected by virus which during the period of blood circulation passed out on to the nasal mucosa and thence by the perineural lymphatics of the olfactory nerve to the subdural space. The evidence presented does not finally exclude a slow "growth through" the hemato-encephalic barrier as an alternative method of infection of the central nervous system, but in the monkey the virus does not pass directly from the blood into the cerebrospinal fluid. An argument is advanced to show the probable close resemblance of the virus of equine encephalomyelitis to certain other viruses capable of exciting nervous disease, viz., those of vesicular stomatitis, yellow fever and louping ill. As a group these viruses probably have "pantropic" affinities, but as they differ materially in their cellular affinities and mode of action from such pantropic viruses as pseudorabies, B virus and herpes they must be relegated to a separate group.

FROM THE AUTHOR'S CONCLUSIONS.

EXPERIMENTAL DIPHTHERIC PARALYSIS. G. RUELLE, Ann. Inst. Pasteur **54**:185, 1935.

Injection of modified diphtheria toxin into the muscles of guinea-pigs induced paralysis, the evolution and intensity of which were influenced by the quantity of toxin used. Antitoxin could be demonstrated in the blood, and it thus appeared that the toxin was fixed by the nervous system before sufficient antitoxin was produced to neutralize it. Antitoxin produced later did not affect the development of paralysis. These observations are in accord with reports of cases of paralysis apparently following unrecognized diphtheria. Although such patients have abnormal quantities of antitoxin, the origin of the condition may still reasonably be diphtheria.

M. S. MARSHALL.

TUBERCULOSIS DUE TO INTRAPARTUM ASPIRATION OF BACILLI. E. KRAUS, Centralbl. f. allg. Path. u. path. Anat. **63**:274, 1935.

The point is made that everything which occurs to a child before severance from the mother is congenital. A child can therefore be considered to have congenital tuberculosis if the infection occurred from aspiration of tubercle bacilli in the birth canal.

The report concerns a mother and child who had tuberculosis. The mother was 26 years old, had open pulmonary tuberculosis and caseous endometritis and died twenty-five days after the birth of her child. The child, a girl, who was

delivered with occiput posterior, succumbed to pneumonia at the age of 18 days. She was 41 cm. long, and weighed 1,700 Gm. at necropsy. She had caseous pneumonia of all lobes of the lungs and caseous tuberculous tracheobronchial lymphadenitis. Similar caseous lesions were found in the spleen, liver and kidneys. Acid-fast bacilli that were morphologically like tubercle bacilli were recovered from the lesions.

The course of events is pictured as follows: Numerous tubercle bacilli were discharged into the birth canal from the caseous foci in the endometrium. During passage through the canal the child breathed and aspirated these bacilli along with amniotic fluid and meconium. The tuberculous pneumonia initiated the caseous tuberculous lymphadenitis, and from this source the bacilli got into the blood stream and caused focal tuberculosis in the kidneys, spleen and liver. The absence of generalized miliary tuberculosis indicated such a course of events and ruled out infection via the placenta.

GEORGE RUKSTINAT.

TESTING OF SMALLPOX VIRUS BY INJECTION INTO THE PLANTAR SKIN OF GUINEA-PIGS. H. O. MÜNSTER, Zentralbl. f. Bakt. (Abt. 1) **133**:412, 1935.

The intracutaneous injection of smallpox virus into the plantar skin of albino guinea-pigs leads to a specific infiltration of the tissue, the extent of which is correlated with the strength of the virus. This method of testing the virus offers the advantages of economy and simplicity. The results seem to be as reliable as those obtained when rabbits are used in the titration of the virus.

PAUL R. CANNON.

THE VIRUS OF MEASLES. T. TANIGUCHI AND OTHERS, Jap. J. Exper. Med. **13**: 577, 1935.

It is believed that a specific virus of measles has been isolated in rabbits by intratesticular inoculation of nasopharyngeal washings and blood from patients with this disorder. Typical measles has followed the injection of the virus in susceptible persons. The virus is killed by the serum of persons convalescent from measles. Minute dotlike bodies, measuring about 0.25 micron in diameter, have been observed in smears from the corneas of infected rabbits. The virus has been cultivated on the chorio-allantoic membrane of the embryo of the chick.

Immunology

ACTIVE IMMUNIZATION AGAINST EXPERIMENTAL POLIOMYELITIS. P. K. OLITSKY and H. R. COX, J. Exper. Med. **63**:109, 1936.

The results obtained in this investigation indicate that virus treated with tannin, sodium ricinoleate or solution of formaldehyde U. S. P. does not satisfactorily immunize monkeys against experimental poliomyelitis.

FROM THE AUTHORS' CONCLUSION.

GROUP SPECIFIC A SUBSTANCE IN HORSE SALIVA. K. LANDSTEINER, J. Exper. Med. **63**:185, 1936.

A method is described for the purification of the A substance isolated from horse saliva, and additional observations on the chemical properties of the preparation are reported. The substance isolated was highly active serologically. It appears to be a polysaccharide.

FROM THE AUTHOR'S SUMMARY.

IMMUNIZATION OF GUINEA-PIGS WITH THE VIRUS OF EQUINE ENCEPHALOMYELITIS. P. K. OLITSKY and H. R. COX, J. Exper. Med. **63**:311, 1936.

A quantitative basis has been established for comparing the immunizing capacities of preparations employed in experiments on equine encephalomyelitis in guinea-pigs.

FROM THE AUTHORS' CONCLUSIONS.

Cross Reactions of Immune Serum to Azoproteins. K. Landsteiner and J. van der Scheer, J. Exper. Med. **63**:325, 1936.

The phenomena observed on absorption of serums immune to azoproteins with heterologous azo-antigens lead to the conclusion that in general these immune serums do not contain an antibody but contain antibody fractions somewhat different in their reactivity for heterologous antigens. From the constitution of the azocomponent it follows that these fractions cannot be specific for distinct chemical groups in the molecule. In fact, inhibition experiments showed that in the cases examined the various antibodies are all specifically directed toward the whole molecule of the homologous hapten. From the Authors' Summary.

Agglutinins for Sheep and Rabbit Erythrocytes in Human Serum. C. A. Stuart, J. Tallman and E. G. E. Anderson, J. Immunol. **28**:75, 1935.

From a study of 760 human serums on which they made the Wassermann test, about 10 per cent of which were syphilitic, Stuart and his associates conclude that there is an apparent relation between the titers of agglutinins for the erythrocytes of the sheep and the rabbit. It appears from a table that a fairly constant relationship is particularly evident in the titers obtained at 5 C., while at 37 C. the rise of the sheep titers is very much faster and out of proportion to that of the rabbit titers. A relationship was also shown to exist between the sheep titers obtained at 37 and at 5 C. The agglutinins for sheep and rabbit erythrocytes were higher in the serums of women than in those of men. I. Davidsohn.

Sheep and Rabbit Cell Agglutinins in Horse Serum Sickness and Infectious Mononucleosis. C. A. Stuart, J. Tallman and E. Brintzenhoff, J. Immunol. **28**:85, 1935.

From a comparison of the titers of the agglutinins for the erythrocytes of the sheep and rabbit in seventeen cases of serum disease, Stuart and associates conclude that the increase in agglutinins for sheep erythrocytes due to injections of horse serum does not appear to be in the Forssman antibodies. They found a marked increase in the aggiutinins for rabbit erythrocytes and were able to remove to a considerable extent the agglutinins for sheep erythrocytes by absorption with rabbit erythrocytes. In the serums of patients with infectious mononucleosis the rise of the agglutinins for rabbit erythrocytes was insignificant, and absorption with rabbit erythrocytes altered only very slightly the titer of the agglutinins for sheep erythrocytes. I. Davidsohn.

Complement Fixation with the Type-Specific Carbohydrate of Haemophilus Influenzae Type b. M. Pittman and K. Goodner, J. Immunol. **29**:239, 1935.

The purified specific carbohydrate of the beta type of the influenza bacillus failed to fix complement in the presence of the homologous horse antiserum but fixed it in the presence of the homologous rabbit antiserum. This action is analogous to the behavior of the type-specific carbohydrate of Pneumoococcus. Suspensions of encapsulated bacilli behaved similarly with regard to the horse and rabbit antiserums if their capsules were kept intact through proper precautions in their preparation. I. Davidsohn.

Agglutinins and the Conditioned Reflex. L. M. Kopeloff, N. Kopeloff and E. Posselt, J. Immunol. **29**:359, 1935.

Inoculations of a pneumococcus vaccine into rabbits were accompanied by a conditioning electrical stimulus. After a rest period the conditioning stimulus alone failed to bring about a rise of agglutinins. The diametrically opposite results reported by Metalnikov and others are based on insufficient data, according to Kopeloff and his associates. The determination of the titer of agglutinins by Heidelberger and Kabat's microchemical nitrogen technic (*J. Exper. Med.* **60**: 643, 1934) is recommended. I. Davidsohn.

SCHICK REACTIONS AND SERUM ANTITOXIN IN CHILDREN TREATED WITH DIPHTHERIA TOXOID. C. N. LEACH and G. PÖCH, J. Immunol. **29**:367, 1935.

Each of 215 children in the age group of 2 to 9 years who had given positive reactions to the Schick test received three injections of diphtheria toxoid. From nine to thirty-two weeks later 91.2 per cent of them gave negative reactions to this test. The antitoxin content of their blood at this time was determined by Claus Jensen's method (Die intrakutane Kaninchen Methode zur Anwertung von Diphtherie-Toxin und Antitoxin, Copenhagen, Levin & Munksgaard, 1933): 83.3 per cent showed titers equal to or above 0.01 unit per cubic centimeter of blood serum, and 14.3 per cent, titers of less than 0.01 unit. Three children had titers of less than 0.0005 units.

I. DAVIDSOHN.

SEROLOGIC TESTS WITH AMINO-ACIDS. J. VAN DER SCHEER and K. LANDSTEINER, J. Immunol. **29**:371, 1935.

The method of preparing para-nitrobenzoyl and para-aminobenzoyl derivatives of various amino-acids is presented. Serums from rabbits immunized with these different antigens were tested for specificity by means of precipitation and inhibition tests. The precipitation tests demonstrated a high degree of serologic specificity in the different amino-acids. Distinct cross-reactions occurred only between related amino-acids. The inhibition tests showed somewhat wider ranges of interrelations between leucine, tryptophan, tyrosine and phenylalanine, but in others the specificity was as striking as in the precipitation tests.

I. DAVIDSOHN.

OPSONINS IN THE SERUM OF PATIENTS CONVALESCENT FROM MEASLES AND FROM SCARLET FEVER AND IN PLACENTAL EXTRACT. R. TUNNICLIFF, J. Infect. Dis. **58**:1, 1936.

The pooled serum of patients convalescent from measles may contain a large amount of opsonin for Diplococcus morbillorum, and about the same amount for Streptococcus scarlatinae as placental extract and serum from persons who had measles and scarlet fever years previously (adult immune serum). The pooled serum of patients convalescent from scarlet fever may have a higher opsonic index for Str. scarlatinae than the pooled serum from patients convalescent from measles but not so high an index as immune horse serum. It may have an opsonic index for D. morbillorum about equal to that of adult immune serum and placental extract. Placental extract also may have as large an amount of opsonin for both Str. scarlatinae and D. morbillorum as adult immune serum.

The results of these experiments indicate that the opsonin content of serum from persons convalescent from measles and scarlet fever and of placental extract for D. morbillorum and Str. scarlatinae may account for some of their protective and curative power in measles and scarlet fever.

FROM THE AUTHOR'S CONCLUSIONS.

THE BACTERICIDAL ACTION OF BOVINE WHOLE BLOOD AND SERUM TOWARD BRUCELLA. M. R. IRWIN, B. A. BEACH and F. N. BELL, J. Infect. Dis. **58**: 15, 1936.

Bovine serum normally contains bactericidins for Brucella abortus. Whole blood shows a bactericidal effect slightly less than that obtained with comparable amounts of serum. Individual animals show uniformity in this bactericidal activity at different times, but a difference between animals was noted. Certain animals that had recovered from a previous Br. abortus infection showed a loss in the bactericidal properties of the whole blood as compared with presumably normal animals. Bovine whole blood exerted no bactericidal effect on Brucella suis; the action of bovine serum on this organism was decidedly less marked than its action on Br. abortus.

FROM THE AUTHORS' SUMMARY.

COMPLEMENT FIXATION WITH VACCINIAL ELEMENTARY BODY SUSPENSIONS AND ANTIVACCINIAL RABBIT SERUM. M. H. FINLAYSON, Brit. J. Exper. Path. **16**: 358, 1935.

Suspensions of the elementary bodies of the virus of vaccinia fix complement in the presence of vaccinial hyperimmune rabbit serum. The complement-fixing activity of the suspensions is reduced but not abolished by filtration through Berkefeld V and N filters and through graded collodion membranes of a pore size of 0.15 micron. The complement-fixing activity is removed from the suspensions by centrifugation at high speeds. The antigenic activity is retained in the resuspended deposit of the elementary bodies.

FROM THE AUTHOR'S SUMMARY.

PHAGOCYTOSIS OF BACILLUS TYPHOSUS IN RELATION TO ITS ANTIGENIC STRUCTURE. S. S. BHATNAGAR, Brit. J. Exper. Path. **16**:375, 1935.

The "opsonic" effect of normal serum is due to the combined action of complement and natural O antibody. The "bacteriotropic" effect of immune serum is due to the combined action of complement and immune O antibody. There is no essential difference between "opsonic" and "bacteriotropic" serum activity; both are demonstrable in the absence of complement, though in considerably less degree. The H antibody has no appreciable effect on phagocytosis. Phagocytosis of Bacillus typhosus is intimately associated with its agglutinability by O antibody. Inagglutinable strains are highly resistant while agglutinable strains are highly susceptible to phagocytosis.

FROM THE AUTHOR'S SUMMARY.

BACILLUS TYPHOSUS IN SHANGHAI. R. C. ROBERTSON and H. YU, J. Path. & Bact. **42**:53, 1936.

The observations of Felix and Pitt concerning the association of the virulence of Bacillus typhosus with its resistance to the action of O antibody have been fully confirmed. Highly virulent cultures of typhoid bacilli which were found to be practically inagglutinable with O serum were used as vaccines for the active immunization of mice and were found to give very marked protection. Serum produced by the use of these strains was of value in producing passive immunity. A group of cultures which were found to be less virulent and more readily agglutinated by O serum was studied. These gave inferior results in the production of active and passive immunity. Observations on the demonstration of the Vi antigenic factor are recorded but are not conclusive, as it was impossible to secure a completely nonvirulent culture of B. typhosus H to absorb H factors and thus produce a pure Vi serum. The findings of Felix and his co-workers with regard to the alterations that appear when virulent, O-resistant strains are grown at low incubation temperatures are confirmed. It is found, however, that a temperature of 100 C. for one hour is required to alter the agglutinability of virulent culture suspensions in O serum.

FROM THE AUTHORS' SUMMARY.

THE ASSAY OF ANTIGENS, WITH SPECIAL REFERENCE TO STAPHYLOCOCCUS TOXOID. M. L. SMITH, J. Path. & Bact. **42**:227, 1936.

Staphylococcic toxoid and toxoid-antitoxin floccules when injected into guinea-pigs or rabbits cause the production of antitoxin and render the animals immune to toxin injected parenterally. The rabbits and guinea-pigs of the Hampstead stock do not contain detectable amounts of natural antitoxin. The response to subcutaneous injections of toxoid is better in rabbits than in guinea-pigs. Rabbits frequently produce measurable amounts of antitoxin after one injection, but guinea-pigs rarely do; two spaced injections produce better immunity. In a group of immunized animals the logarithms of the antitoxin produced are symmetrically distributed. The precision with which toxoids can be compared as to relative potencies when different numbers of rabbits or guinea-pigs are used is discussed. The precision of testing increases with the antigenic activity of the toxoid. The relative merits and disadvantages of different methods of standardizing antigens are discussed.

FROM THE AUTHOR'S SUMMARY.

ACTION OF STAPHYLOCOCCUS SECRETIONS. O. GENGOU, Ann. Inst. Pasteur **54**:428, 1935.

Studies of the action of staphylococcus secretion on hematoblasts in connection with the production of postoperative thrombi led Gengou to make the following statement: "The proof of the intervention of bacteria may be demonstrated really only when organisms are isolated from spontaneous postoperative thrombi. But we believe that we have shown that staphylococcus secretions appear to be capable of provoking the agglomeration of cells which constitute the so-called viscous degeneration which is seen in such thrombi. This conclusion appears to us to be a favorable argument for the concept of the infectious origin of the latter."

M. S. MARSHALL.

LIPOID HAPTEN FROM TUBERCLE BACILLI. G. SANDOR and C. NINNI, Ann. Inst. Pasteur **55**:38, 1935.

Aqueous extracts from living tubercle bacilli yielded a lipoid antigen. This complement-fixing hapten appeared to be a substance very easily absorbed which acquired the property of solubility of the other substances contained in the combination. This raised the question in the minds of the authors whether the hapten qualities attributed to protein or similar extracted substances may not be due to complex absorption conditioned by the lipoid hapten.

M. S. MARSHALL.

ALTERATIONS OF THE PHAGOCYTIC ABILITY OF LEUKOCYTES AFTER INJECTIONS OF THORIUM DIOXIDE. A. KOCH, Frankfurt. Ztschr. f. Path. **47**:429, 1935.

Koch tried to determine whether or not diagnostic use of thorium dioxide causes a blockade of the reticulo-endothelial system and diminished resistance. Rabbits were used. Phagocytes were derived from sterile pus produced by applying cantharides plasters to the inside of the rabbits' ears, and streptococci were used as the objects of phagocytosis in vitro. Injections of thorium dioxide caused a temporary decrease of phagocytes. In a splenectomized rabbit which had received thorium-dioxide three years previously no marked deviation in the percentage of phagocytes was noted. Koch therefore concludes that storage of thorium dioxide does not affect the defense mechanism of the body permanently.

OTTO SAPHIR.

THE SO-CALLED BLOOD GROUP ENZYME. F. SCHIFF and F. A. BURÓN, Klin. Wchnschr. **14**:710, 1935.

The more turbid the saliva is, the more blood group enzyme it contains. Saliva that is rich in enzyme has a marked sediment which contains most of the enzyme. The supernatant fluid obtained by centrifugation contains very little of it. If the saliva is allowed to stand, the enzyme increases, and it reaches a maximum when the saliva is kept twenty-four hours at body temperature. The supernatant fluid obtained from such saliva by centrifugation is rich in the enzyme. The authors believe that it is carried by body cells derived from the salivary glands. Parasympathetic stimulation has no effect, but after sympathetic stimulation with epinephrine the saliva contains much of enzyme. Gastric content obtained two days after death was found to contain it; it was also found in the gastric content in a case of achylia.

A. S. WIENER.

THE RESISTANCE OF THE HEDGEHOG TO TETANUS TOXIN. J. V. VÁSÁRHELYI, Zentralbl. f. Bakt. (Abt. 1) **133**:366, 1935.

Vásárhelyi studied the resistance of the hedgehog to tetanus toxin, using approximately fifty animals. He observed that the hedgehog possesses no absolute resistance against this toxin but that the lethal dose injected subcutaneously is

10,000 times the lethal dose for the mouse. The blood serum contains no antitoxin. Tetanus toxin injected subcutaneousy disappears from the blood stream in approximately twenty hours. The brain, pancreas, spleen and leukocytes possess neutralizing properties for it. Tetanus spores injected into hedgehogs were phagocytosed more rapidly than such spores are in the mouse, the rat, the guinea-pig or the rabbit. The author believes that the resistance of the hedgehog to tetanus toxin is correlated with the marked toxin-combining power of certain of its organs and tissues.

PAUL R. CANNON.

THE ISOHEMOLYSINS. T. ANDERSEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:41, 1935.

This report is based on quantitative determinations of isolysins and iso-agglutinins in 395 human serums. Seventy-nine per cent of the serums contained demonstrable isolysins. Serums of the three blood groups varied considerably with regard to the occurrence in them of the isolysins. The anti-A lysin in the groups O and B showed greater frequency (85 per cent) and higher titers than did the anti-B lysin in the groups O and A (62 per cent). A direct relation was noted between the presence of iso-agglutinins in high titer and the occurrence of isolysins. The isolysins are very sensitive to elevated temperature and disappear rapidly on standing. Their absence from some serums is not accounted for by difference in the titer of complement in the serums. The isolysin titers for the different age groups show a rapid rise in childhood and youth, followed by a steady high level until about the thirtieth year for the anti-B isolysin and until about the fiftieth year for the anti-A isolysin, and by a precipitous drop afterward. Andersen suggests the possibility that some of the anti-A isolysin may be due to heterogenetic antigens of bacterial origin.

I. DAVIDSOHN.

THE SO-CALLED BLOOD GROUP FERMENT IN SALIVA. O. SIEVERS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:163, 1935.

In addition to the blood group specific factor, the saliva obtained before cleansing the mouth contains a ferment-like substance that is able to destroy the blood group factor. The ferment is inactive against the blood group factor A that is present in alcoholic extracts of blood, but it destroys the factor in extracts of saliva prepared with 50 per cent alcohol. According to Sievers, the enhanced destructive effect of the ferment on the A factor in Witte peptone as compared with the A factor in saliva is due to the multiplication of the enzyme in the peptone. Broth acted like peptone as a culture medium at body temperature. The ferment was shown to multiply in serial transmission in the peptone and in the broth. The transmission was more regular and extended over a larger series if heated saliva with the A factor was added. The ferment did not pass the Berkefeld filter and was readily absorbed by kaolin. Attempts to isolate micro-organisms that would be responsible for the ferment action failed.

I. DAVIDSOHN.

INACTIVATION OF THE THIRD FRACTION OF THE COMPLEMENT. O. G. BIER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **85**:181, 1935.

Solution of formaldehyde and sodium hydrosulfite inactivated the third component of the complement in fresh as well as in heated serum. Inactivation of the same component occurred in complement that diffused into a suspended strip of filter paper the end of which was dipped into serum. Iodides added to serum did not interfere with the inactivation by diffusion but such admixture of iodides as well as heating the serum prevented the inactivation by yeast. The mechanism of the inactivation by yeast and by the diffusion into filter paper is apparently based on adsorption, but it is controlled by different physicochemical factors.

I. DAVIDSOHN.

Tumors

GROWTH OF ROUS SARCOMA INOCULATED INTO THE BRAIN. E. VAZQUEZ-LÓPEZ, Am. J. Cancer **26**:29, 1936.

Intracerebral growth of the Rous sarcoma was produced both by implantation of tissue grafts and by injection of cell-free material (filtrates or desiccated extracts). When grafts are implanted, the tumors grow at the expense of the transplanted tissue, forming well limited nodules without invasion of the surrounding nerve tissue, which is compressed and displaced. When cell-free material is inoculated, the tumor grows diffusely, invading the brain by cellular infiltration, especially along the perivascular Virchow-Robin spaces. In the first stages of development the tumor is constituted of accumulations of compound granular corpuscles derived from microglia cells and similar to those commonly produced in all pathologic processes of the nervous parenchyma. Later the tumor cells appear among these elements. The tumor evokes a strong neuroglial reaction in all the surrounding area. The microglia reacts by migration and accumulation throughout the periphery of the neoplasm. This response is more conspicuous in tumors produced by cell-free material. There are morphologic, topographic and tinctorial relations between the microglial cells and their derivatives on the one hand and the ameboid elements of the Rous sarcoma on the other, which force the conclusion that they are identical. The fact that the tumor develops in its early stages at the expense of the compound granular corpuscles (derived from microglia) and the relations of the microglia with the ameboid cells of the tumor compel one to suppose that the microglial response, at least in the advanced stages, is probably not defensive but provoked by a stimulus arising from the free sarcoma agent, which acts always on cells with characters similar to those of the microglia.

FROM THE AUTHOR'S SUMMARY.

RELATION BETWEEN MAMMARY CANCER AND THE SEXUAL CYCLE IN MICE. E. L. BURNS, M. MOSKOP, V. SUNTZEFF and LEO LOEB, Am. J. Cancer **26**:56, 1936.

There is no noticeable parallelism between features of the sexual cycle and the frequency with which cancer appears in these strains. It may be concluded, therefore, that the hereditary tendency to acquire cancer, which is characteristic of strains of inbred mice, is not due essentially to the nature of the sexual cycle which distinguishes these strains from each other.

FROM THE AUTHORS' SUMMARY.

EXPERIMENTAL PRODUCTION OF TERATOMA TESTIS IN THE FOWL. H. J. BAGG, Am. J. Cancer **26**: 69, 1936.

Zinc chloride when injected into the testes of adult roosters will produce teratoma testis in a small percentage of the birds. Under the conditions of the experiment these tumors occur only if the injections are given when gonodal activity is pronounced, i. e., in the first three months of the year. The adult testis may be stimulated to growth and functional activity by prolonged injection of hormones, especially the alkaline extract of the anterior lobe of the pituitary gland of the sheep. Such a testis may be so conditioned that the irritation of the injected zinc chloride will result in the formation of a testicular teratoma, which may appear as late as the end of August. When this method was applied to immature testes it did not result in new growth in the experiments recorded here. The prolonged injection of gonadotropic hormones alone in young and in adult birds without chemical irritation has given no neoplastic growth. The four tumors described have shown rapid growth; no metastasis has been noted, and the tumors have not grown on transplantation. These findings are apparently associated with the adult type of histologic structure, which showed cartilage and bone, muscle, fat, connective and nerve tissue, glands and epithelium. Feather follicles and areas of carcinoma and adenocarcinoma were also noted.

FROM THE AUTHOR'S CONCLUSIONS.

DI-BENZANTHRACENE TUMORS IN CONTROLLED STRAINS OF MICE. C. F. BRANCH, Am. J. Cancer **26**:110, 1936.

When 1, 2, 5, 6-di-benzanthracene was painted on the depilated skins of high and low cancer strains of mice, almost twice as many cutaneous tumors developed in the low cancer strain as in the other. No such proportion was observed in the few inoculated animals in which sarcoma developed. In an otherwise normally high cancer strain painting with 1, 2, 5, 6-di-benzanthracene seemed materially to reduce the incidence of spontaneous tumors in breeding females, but untreated breeding controls of the same strain also showed a very low incidence of spontaneous tumors. Blocking with trypan blue had no appreciable effect on the carcinogenic activity of 1, 2, 5, 6-di-benzanthracene. In spite of the positive identification of 1, 2, 5, 6-di-benzanthracene in the gastro-intestinal and genito-urinary tracts of treated mice no instance of a new growth in these systems was encountered.

FROM THE AUTHOR'S SUMMARY.

AN INTERSTITIAL CELL TUMOR OF THE TESTIS WITH HYPERGENITALISM. C. A. STEWART, E. T. BELL and A. B. ROEHLKE, Am. J. Cancer **26**:144, 1936.

An interstitial cell tumor of the testis with hypergenitalism in a boy 5 years of age is reported. Pubic hair was present, the penis was greatly enlarged, and the testis containing the tumor had developed almost to the point of formation of spermatozoa. It is established that the secretion of the interstitial cells is important in the development of the secondary sexual characteristics of the male.

FROM THE AUTHORS' SUMMARY.

PRIMARY TUMOR OF THE HEART. J. M. REEVES and PAUL MICHAEL, Am. Heart J. **11**:233, 1936.

A malignant tumor (rhabdomyosarcoma) was found in the heart. As in other reported cases of primary tumor of the heart, the site of origin was probably in the auricles. The ventricular walls on both sides showed microscopic infiltration but grossly appeared uninvolved. Death occurred from erosion of the left auricular wall with fatal exsanguination. This apparently was a slow erosion, with hemorrhage into the walls of the auricle as shown by the stasis of blood between the tumor fibers. Some of the spindle cells which appeared to be less embryonic showed areas of cross-striation. Mitoses were present, and giant cells were fairly common. Diligent search failed to reveal any evidence of tumor elsewhere

FROM THE AUTHORS' SUMMARY.

ARSENIC AS AN ETIOLOGIC AGENT IN CERTAIN TYPES OF CARCINOMA. H. MONTGOMERY, Arch. Dermat. & Syph. **32**:218, 1935.

Montgomery reviews 85 cases of arsenical keratosis in which were found: the arsenical type of carcinoma, 16; the arsenical type of carcinoma simulating Bowen's disease, 2; Bowen's disease, 5, and superficial carcinomatosis, 41. The arsenic was determined by Osterberg's chemical method and Osborne's histologic method.

Arsenical carcinoma is characterized clinically by multiplicity of lesions varying from seborrhea-like crusts to lesions several centimeters in diameter covered with thick tenacious hard crust, verrucous and dirty, the removal of which reveals a wide rolled pearly border and appreciable induration. It is usually of the squamous cell type. This is in contrast to the superficial threadlike border of multiple superficial carcinomatosis. The author adheres to the concept of Bowen's disease as a clinical picture and not a histologic process as evidenced by microscopic examination. The process represented by the term "Bowen's disease" also occurs in precancerous conditions, such as keratoses in old people and those caused by roentgen rays, tar and arsenic. It is difficult to prove the etiologic relationship of arsenic to carcinomatosis. Apparently there is a factor of individual sensitivity or idiosyncrasy to the drug.

S. W. BECKER.

THE RELATION OF LEUKOSIS TO SARCOMA OF CHICKENS. J. FURTH, J. Exper. Med. **63**:127 and 145, 1936.

Mixed Osteochondrosarcoma and Lymphomatosis.—Osteochondrosarcoma developed in a chicken inoculated with leukosis of strain 2. In successive passages made by intramuscular implantation of tumor tissue (strain 12) derived from this chicken there occurred (*a*) osteochondrosarcoma free from leukosis or (*b*) leukosis free from osteochondrosarcoma or (*c*) osteochondrosarcoma in association with leukosis. The malignant sarcoma cells of this strain have the morphologic characteristics of osteoblasts. Implantation of tumor tissue from chickens with lesions (*a*) and (*c*) yielded both osteochondrosarcoma and leukosis. Successive passages made by intravenous and intramuscular injections of blood from chickens with leukosis yielded leukosis only. Hence it is very probable that strain 12 consists of a mixture of an osteochondrosarcoma-producing agent and the agent of leukosis of strain 2. Strain 12 is readily transmitted by material containing the living malignant osteoblasts, but transmission is seldom successful with virus freed from living cells. This virus produces neoplasms only when brought in contact with bone or cartilage.

Sarcomas of Strains 11 and 15 and Their Relation to Leukosis.—Two transmissible sarcomas are described. Strain 15 originated in a chicken given an injection of material showing neurolymphomatosis of strain 5. Its virus produces sarcoma characterized by peculiar giant cells, different from those of the other known chicken sarcomas. It does not produce neurolymphomatosis or leukosis. Strain 11 is morphologically similar to the spindle cell sarcoma of Rous and of other workers. It occurred in an untreated control chicken, and its virus produces sarcoma unassociated with leukosis. Both strains 11 and 15 can be readily transmitted with material free from viable cells, and their virus can be preserved by drying in the frozen state. The viruses of leukosis of strain 1 and sarcoma of strain 11 when injected into the same bird retain their identity and can be readily reisolated.

FROM THE AUTHOR'S SUMMARIES.

STRAIN OF INBRED MICE (CBA) WITH LOW TUMOR INCIDENCE. L. C. STRONG, Brit. J. Exper. Path. **17**:60, 1936.

The female mice of an inbred strain of mice (CBA) are relatively resistant to spontaneous carcinoma of the mammary gland up to eighteen months of life. This applies to females that have been used for a moderate amount of breeding and kept on the diet used in this experiment (rolled oats, meat scrap, powdered whole milk and salt). Visceral tumors, including adenoma of the liver, may be expected in female mice of this strain.

FROM THE AUTHOR'S SUMMARY.

FLOCCULATING POWER OF CANCER SERUM. M. L. CHEVREL-BODIN, F. CHEVREL and M. CORMIER, Ann. Inst. Pasteur **55**:108, 1935.

The serums of a considerable proportion of cancerous persons have a high flocculating power for alcoholic extract of organs of the Bordet-Ruelens type. This power is not manifested if the extract of organs (cancerous or not) is diluted in a solution of sodium or potassium chloride. It is, however, evident if the organ extract is diluted in a solution of calcium chloride or acetate.

The flocculating power appears late and seems to correlate with generalization of the cancerous process. This property being common to cancerous and syphilitic serums, it is permissible to believe that in the course of cancer and of syphilis the blood undergoes comparable modifications.

FROM THE AUTHORS' CONCLUSIONS.

DIAGNOSIS OF CANCER BY EXACT CHEMICAL MEASUREMENTS. R. LINKS, Ztschr. f. Krebsforsch. **41**:166, 1934.

There is presented a method of diagnosis which Links claims is suited to the recognition of early cases of cancer by changes in the serum. He finds that during

the process of clotting there are quantitative changes in the serum with progressive variation of its constituents. He determined the varying concentrations of potassium and magnesium salts, and for the interpretation of his findings used a formula in which one factor is the reciprocal of the hemoglobin content of the blood. A priori, this would appear to be the determining factor in his results, but despite this they show a striking predominance of correct findings. H. E. Eggers.

Carcinoma at Early Ages. H. Rosenberg, Ztschr. f. Krebsforsch. **41**:230, 1934.

In a study of cases of juvenile cancer observed at the Pathologic Institute of Munich during the years from 1910 to 1933, 81 cases, 2 per cent of all the cases of cancer during this period, were observed. These cases involved principally the gastro-intestinal tract. Cancer of the female genital organs was rare under the age of 25 years, but in the period from 26 to 30 years it was almost as frequent relatively as in later life. Diagnostic errors were little more frequent in these cases than in those occurring at later ages. Nothing could be noted to indicate exceptional malignancy of the early cancer. Cohnheim's theory was applicable only rarely. H. E. Eggers.

The Fuchs Cancer Reaction. V. Kafka Jr., Ztschr. f. Krebsforsch. **41**:369, 1934.

Kafka obtained correctly positive results with the Fuchs reaction in 91 per cent of fifty-six cases of cancer; there was one case in which the reaction was doubtfully positive and four cases in which it was erroneously negative. Erroneously positive reactions in cases in which cancer was not present were not observed. However, in three cases of sarcoma he obtained one correctly positive result.

H. E. Eggers.

Primary Carcinoma of the Seminal Vesicle. W. Trachsler, Ztschr. f. Krebsforsch. **41**:382, 1934.

The cancer was of the small, undifferentiated cell type. It infiltrated the adjoining left lobe of the prostate, and there were renal and vertebral metastases. Clinically there was localized vertebral pain which was noted after a trivial injury, then, after a long remission, the appearance of spinal deformity (Kümmell's disease). H. E. Eggers.

Society Transactions

BUFFALO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, May 27, 1936

KORNEL TERPLAN, *President*

WILLIAM F. JACOBS, *Secretary*

ANGIOMATOSIS OF THE BREAST. S. SANES.

Two cases of angiomatosis of the breast are reported for several reasons: It is a rare disease. In both patients clinical observations were carefully made from a time prior to the appearance of the primary lesion until death. Diagnostic studies were completed with postmortem examinations. Clinically and pathologically the cases simulate each other so much as to suggest almost that they represent a distinct entity. In retrospect, their course, the histologic observations and the outcome offer lessons that can be practically applied to the prognosis and treatment of the condition in future instances. From a general pathologic standpoint this report attempts to add some evidence to the clarification of problems that often arise in discussions on "angioma," "systemic angiomatosis," "metastasizing angioma" and "malignant angioma."

CASE 1.—A white woman 49 years of age was first admitted to the hospital in 1927 for removal of an acutely inflamed appendix. When she was next seen, in 1931, she complained of a lump in the left breast of three weeks' duration. This lump was walnut-sized, tender, attached to the overlying skin and firm. The axillary nodes were not palpable. The right breast was not remarkable. Grossly the lump, when excised, was a nodule with gray-red areas. The histologic diagnosis was a typical hemangiomatous formation with adenomatous hyperplasia.

Examination in the spring of 1933 revealed no recurrence. The right breast was still normal. Several months later, however, a lump appeared in the right breast. Grossly, it was considered a walnut-sized cavernous angioma. The histologic description read "angiomatosis of the breast with rather diffuse proliferation of newly formed vessels, some of which show cystic dilation. A few large vessels are thrombosed. In some of the cystic structures there is pseudopapillary growth of endothelium. Less differentiated areas with a tendency toward diffuse endothelial proliferation are also present." In April 1935 the patient was again admitted to the hospital for tender nodules along the operative scars and on the back. One of these proved to be histologically an angioblastic tumor situated in the subcutis. The vessels were of the capillary type. A few less differentiated areas with less distinct capillary formation were seen. With further study, lesions were found in the wall of the chest, the ribs and the bones of the pelvis and the left leg and in the lungs. At autopsy, angiomatous tumors were seen in the skin, muscles, lungs, lymph nodes, mesentery, omentum, epicardium, liver, spleen and bones of the pelvis.

CASE 2.—A white woman 28 years of age was first admitted to the hospital in 1927 for acute appendicitis. A second admission was sought in August 1934 because of a lump in the right breast of four months' duration. This lump was orange-sized, nodular, firm and movable. The nipple was slightly retracted. The superficial veins were dilated. The entire breast was excised subcutaneously. It showed an apparently well encapsulated angiomatous tumor. Histologically the tumor was diagnosed as angiomatosis of the breast with many small capillary vessels but also with less differentiated areas of endothelial proliferation. Within

the next year lesions developed in the skin, scalp and bones. In addition to the tumors at these sites, angiomatous tumors were demonstrated at autopsy in the lung, liver, kidney and both ovaries.

PRODUCTION OF TRUE SARCOMA AND CARCINOSARCOMA BY LONG LASTING ULTRAVIOLET IRRADIATION OF RATS AND MICE. WALTER PUTSCHAR.

Roffo has shown that by ultraviolet irradiation, besides epithelial blastomas, new growths of sarcomatous character can be produced. Since, however, histologically less differentiated epithelial blastoma may simulate sarcoma to a very high degree, it is necessary to prove the mesenchymatous nature of the experimental sarcoma by demonstrating differentiated mesenchymatous structures between the tumor cells. In experiments which I carried out in collaboration with Holtz of Berlin it was possible to produce in one rat a spindle cell sarcoma with formation of collagenous fibers and in another rat a chondrosarcoma originating from the cartilage in the ear. In other instances carcinomatous and sarcomatous structures were found completely intermingled (so-called carcinosarcoma). These tumors were especially observed in the marginal areas or the so-called contact zone between two adjacent blastomas. In these restricted areas carcinoma and sarcoma had apparently grown independently of each other under the stimulating effect of the ultraviolet radiation on the epidermal and dermal cells.

PRIMARY CARCINOMA OF THE LIVER OF TYPICAL PERITHELIOMATOUS STRUCTURE. K. L. TERPLAN.

This case is presented because it took some time to recognize the carcinomatous nature of the tumor behind many deceiving typical peritheliomatous structures.

A 57 year old white woman felt perfectly well until two months prior to examination. She had been treated for obesity by a masseuse, who discovered a painless mass below the right costal margin. Only occasionally the patient complained of a "filled" feeling, some eructations and nausea. The laboratory examinations gave entirely negative results. At laparotomy a large hemorrhagic tumor about the size of a coconut was found suspended on a broad pedicle from the anterior margin of the right lobe of the liver. Three days after operation the patient died of pneumonia. On biopsy the histologic diagnosis had been "a typical perithelioma." At postmortem examination the liver showed a small grapefruit-sized cyst filled with bloody material and necrotic tumor tissue and lined by a thin coat of grayish yellow tumor. This large cyst was in the right lobe. There were several pea to plum-sized nodules in both lobes, most of them grayish white, some of them very vascular. The parenchyma of the liver around and between the tumor nodules appeared entirely normal. No metastases were found outside the liver. Detailed histologic studies of the tumor tissue, especially of small nodules surrounded by normal liver parenchyma, disclosed the epithelial character of the blastoma cells in this peculiar peritheliomatous arrangement. Inasmuch as direct transformation of liver cells into these large spindle-shaped and high columnar cells arranged in rosette-like structures around very distinct capillaries could be seen, the epithelial character of the tumor was proved. This case, therefore, is apparently analogous to that of L'Esperance, reported from Ewing's laboratory, known under the name of peritheliomatous hemorrhagic hepatoma.

Book Reviews

A Textbook of Pathology. W. G. MacCallum, Professor of Pathology and Bacteriology, the Johns Hopkins University, Baltimore. Edition 6. Cloth. Price, $10. Pp. 1277, with 697 illustrations. Philadelphia: W. B. Saunders Company, 1936.

This book was published first in 1916. The present edition represents an especially thorough revision in order to give proper account of recent advances, particularly in the knowledge of endocrine disturbances, vitamin deficiencies and virus diseases. The general plan of the book remains unchanged. The fundamental principle of the plan is described clearly in the following paragraph from the preface: "Beginning, then, with the idea that all departures from normal health are brought about by some harmful or disturbing agency, the endeavor has been made to trace these changes back to their cause, and then to describe not only the anatomical alterations but the disturbances of function and the reaction which tends to restore the body to a normal state and even to establish a protection against a recurrence of the same injury. If this, with some mention of symptoms makes the book seem like a treatise on clinical medicine, it is only because pathology and clinical medicine are, after all, the same thing viewed from slightly different angles." First come chapters on disturbances of the fluids of the body; on cellular structure and metabolism; on disturbances of nutrition and metabolism. Then the defenses of the body against injuries of various kinds are described, with discussions of the inflammatory reaction and of reparative processes, followed by chapters on the various types of injuries—mechanical, chemical, bacterial and the other forms of infection. Under the types of injury caused by infectious agents are described in detail the changes and other results of individual infectious and parasitic diseases. Now follow chapters on the effects of injuries on the blood and blood-forming organs and on the organs of internal secretions, on diseases related to specific dietary deficiencies and on disturbances of lipoid metabolism. The remaining chapters are devoted to conditions of unknown etiology affecting the central nervous system, muscles and bones, including arthritis deformans; diseases of the teeth and related structures, and tumors. The chapters on tumors occupy 200 pages. Within the frame thus outlined is a remarkably competent and comprehensive presentation of the topics grouped under general and special pathology in the textbook of the traditional scheme. MacCallum's plan is logical and leads to synthetic organization of the material with respect to the body as a whole. The illustrations are uniformly of the best grade. The index is complete. The references at the end of chapters cite "readable and comprehensive papers which review the subject and give further and more complete references." The style is clear, direct, exact and pleasant, especially in descriptive passages. MacCallum's book will remain a leader in its class.

Bacterial Nutrition: Material for a Comparative Physiology of Bacteria. By C. J. G. Knight. Medical Research Council, Special Report Series, No. 210. Paper. Price, 3 shillings. Pp. 182. London: His Majesty's Stationery Office, 1936.

Knight states that the aim of this report is to collect and systematize the available observations on bacterial nutrition, particularly with reference to organisms of medical interest, and to unify these observations on the basis of a consistent theory which shall be a practical guide for the further study of the nutrition of disease-producing bacteria.

The first and longest part of the report deals with a review of the literature concerning bacterial nutrition. This section is subdivided into considerations of the

general conditions of growth, photosynthesizing bacteria, chemosynthesizing autotrophic bacteria, and heterotrophic bacteria. The author logically deals with heterotrophic bacteria of special medical interest, as well as other heterotrophic bacteria, according to the complexity of their nutritive requirements. The review of the literature is adequate and up to date. Approximately four hundred and forty references are cited.

The second part of the monograph deals with the data presented in the first part in the light of the hypothesis that the different types of nutritive requirement exhibited by bacterial species are criteria of the successive levels of the bacteria in an evolutionary scale. At the bottom of this scale are those bacteria that grow and multiply at the expense of relatively simple food substances. The higher forms have more elaborate requirements and are dependent on a more specialized environment. Parasitism is a characteristic of those forms that either have more elaborate requirements or have lost some power of synthesizing necessary compounds from simple sources.

The third part of the report is concerned with the ways in which bacteria can adapt themselves to changes in nutritional requirements.

The author frequently uses the trinomial nomenclature for bacteria, a practice usually discouraged in this country.

The monograph is exceptionally well organized; the work of other investigators is clearly evaluated, and the argument is smooth and logical. It is a valuable contribution to the nutritional aspect of bacterial physiology and fills a need felt for some time. The high quality of the report is typical of the Special Report Series of the Medical Research Council.

Chemical Procedures for Clinical Laboratories. By Marjorie R. Mattice, A.B., Sc.M., Assistant Professor of Clinical Pathology, New York Post-Graduate Medical School of the Columbia University; Assistant Director of the Biochemical Laboratory, New York Post-Graduate Hospital; Consultant Chemist, Reconstruction Hospital, New York City. Cloth. Price, $6.50. Pp. 520, with 90 engravings and 2 colored plates. Philadelphia: Lea & Febiger, 1936.

An outgrowth of ten years' teaching, this book is a thorough, concise and clear presentation of the chemical aspects of clinical laboratory procedures. Only such data are incorporated as have proved analytically practical. The clinical application and the chemical discussions are tersely but lucidly presented. The bibliographic data are few, occurring in situ. The directions are detailed and exacting, and descriptions of many very recent methods, such as the Lueder-Hollander system for estimation of duodenal enzymes and the McCullagh method for the determination of blood iodine, are inserted. The subject matter falls into five groups: blood determinations, urinalyses, tests on gastro-intestinal secretions, analyses of miscellaneous bliologic fluids and the preparation of reagents. The determination of metallic poisons is included under urinalysis, directions also being given for blood, feces, etc. Chapter 24 contains much useful information pertaining to the chemistry of transudates and exudates, synovial fluid, pericardial fluid, amniotic fluid, hydrocele fluid, spermatocele fluid, seminal plasma and sweat. Particularly valuable is the appendix, consisting in large part of a résumé of normal data, details of the use of the colorimeter, mathematical notes and calculations and directions for the preparation of reagents. The chapters on the acid-base balance of the blood and the nonprotein nitrogen and its components are notably excellent. Mattice's book will find a favorable place in the laboratory and medical library.